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SEX PROBLEMS IN ALGAE, FUNGI AND PROTOZOA

A Critical Account Following the Review of R. A. Lewin

MAX HARTMANN

Tübingen, Germany

In a symposium on "Sex in Microorganisms" Lewin (1954) has given a review on sex in unicellular algae. In this report my thesis of a general bipolar bisexuality and a bisexual potency of isogamous haplomonoecious algae (generally called by Lewin homothallic according to the somewhat improper expression of Blakeslee, in spite of repeated objections by Correns, Kniep and myself) is rejected as a "theoretical anthropomorphic concept." Also, the existence of two physiologically different sorts of gametes receives an unfavorable and insufficient evaluation because of some negative statements by the botanists Czurda, Mainx, Pascher and Pringsheim at Prague. These statements have already been refuted in detail. Evidence for the existence of physiologically diverse gametes has often been provided by the method of residual gametes, a method due originally to Haemmerling not, as Lewin apparently believes, to Moewus. Furthermore the indication for sex-specific agents (gamones) in gametes of algae is questioned, obviously since Lewin seems to believe that sex substances in unicellular algae have been mentioned only by Moewus "whose published results contain a number of discrepant features." For the same reason the existence of relative sexuality is doubted. Actually, the term "gamone" for sex-specific agents did not originate with Moewus, as Lewin seems to assume, but was proposed by Hartmann and Schartau (1940) after consultation with Prof. R. Kuhn.

This rather partial presentation of sexuality in unicellular algae cannot pass without contradiction and calls for critical adjustment. It is impossible to give a correct critique of positive and negative findings concerning bipolar bisexuality, bisexual potency, relative sexuality, and the occurrence of specific sex substances, or gamones, in fertilization by limiting the discussion to unicellular algae only. It is necessary to pay regard also to the findings in multicellular algae (especially *Ectocarpus siliculosus*), fungi and protozoa. I claim to be entitled to make such a criticism of Lewin's

report not only on account of my own investigations, which extend over decades, and those of my collaborators and students with regard to sex in algae, but also by the fact that my present collaborators, Foerster and Wiese, have, at my suggestion, accomplished the most careful and thorough examination yet made of the statements by Moewus on sexuality in *Chlamydomonas eugametos* and on its gamones in particular. In agreement with American investigators, they could not verify the assertion of Moewus on the carotinoid nature of the gamones in this species. Moreover they showed that the cited statements of Moewus are wrong and why. They proved the existence of gamones in *Chlamydomonas eugametos* and *C. reinhardi*, determined the specific effect of the gamones, and showed them to be proteins with sugar components. In the following account the points at issue will be treated under three subjects:

1. Bipolar bisexuality in isogamous monoecious algae as indicated by the residual gamete method, and in isogamous and autogamous protozoa (flagellates), as indicated by the cytological findings of Cleveland.
2. Indication of relative sexuality in algae, fungi and protozoa.
3. Indication of gamones in algae and fungi.

BIPOLAR BISEXUALITY IN ISOGAMOUS HAPLOMONOECIOUS ALGAE AND PROTOZOA

Algae. Haemmerling (1934) has since 1931 investigated the reproduction and sexuality of the Dasycladacean, *Acetabularia mediterranea.* It is well known that his work led to valuable results, e.g. the demonstration of the mononuclear structure and the diploidy of this alga and especially the first proof for the dependence of form on substances produced by the nucleus and the cytoplasm. In the present review only his findings in connection with sexuality and the method of residual gametes, which he developed, are of interest.

Sex determination in Acetabularia is diplophaenotypic and independent of the reduction division. The sex is determined at the latest for single cysts. This indicates that gametes of some cysts are plus while those of others are minus. In case only gametes from cysts of the same sex of any one plant are released, the plant seems to be dioecious for the combination of gametes from different plants shows a "Zweierschema." On the other hand, if gametes from cysts with different sex are released from the same plant, pairing between gametes takes place and accordingly Acetabularia proves to be monoecious. In this case there remains after copulation a smaller or bigger number of the strongly phototactic gametes which gather in the different Boveri-dishes near the source of light and can be isolated. These residual gametes copulate in new combinations and prove to be sexually differentiated according to a bipolar scheme. This bipolar sexual differentiation of the residual gametes is further confirmed by the fact that one type of residual gamete is able to mate only with a plus type or only with a minus type of gamete from a different cyst (table 1). This method of Haemmerling was applied with success by Moewus (1933), under control of Dr. Haemmer-

ling, to the haplomonoecious isogamous *Stephanosphaera pluvialis* and similarly haplomonoecious isogamous strain of *Protosiphon botryoides*. Both of these monoecious algae form two different sorts of gametes, i.e. plus and minus, or male and female, as proved by the residual gamete method. These results of Haemmerling and Moewus have been discussed earlier (Hartmann, 1932).

At the same time Dr. Rosenberg (see Hartmann, 1932) applied the residual gamete method in her investigations on clone cultures of the haplomonoecious isogamous alga Hydrodictyon with results like those of Haemmerling. Ob-

TABLE 1

ACETABULARIA MEDITERRANEA (CHLOROPHYCEA), "ZWEIERSCHEMA." RESIDUAL GAMETE CROSSES, INCLUDING ONE + DIOECIOUS PLANT. Z = COPULATIONS AND FORMING OF ZYGOTES, O = NO REACTION. AFTER HAEMMERLING, 1934.

20.II 31	+ mon. I_1	+ mon. II_3	+ dioc II_4	− mon. I_3	− mon. II_4	− mon. II_5	− mon. II_7
+ mon. I_1	O	O	O	Z	Z	Z	Z
+ mon. II_3	O	O	O	Z	Z	Z	Z
+ dioc II_4	O	O	O	Z	Z	Z	Z
− mon. I_3	Z	Z	Z	O	O	O	O
− mon. II_4	Z	Z	Z	O	O	O	O
− mon. II_5	Z	Z	Z	O	O	O	O
− mon. II_7	Z	Z	Z	O	O	O	O

servations on this same alga had induced Mainx (1933) to agree with Czurda's (1932) conception that there exists but one type of gamete, which is said to be male first and to turn female afterwards. This interpretation of Czurda and Mainx was thus refuted by the investigations of Rosenberg on the same organism chiefly used by Mainx. The facts are plainly the reverse of what they are made to appear in the account of Lewin.

Later Lerche (1937) proved several strains of Dunaliella (*D. parva*, *D. persei*, *D. euclora* and *D. minuta*) to be monoecious, while *Dunaliella salina* was haplogenotypically dioecious. On account of difficulties with the application of the residual gamete method for these organisms, she used it only in her investigations on the related *Haematococcus pluvialis* and with the same result as described above for other monoecious algae. Of special interest are her findings regarding the pairing of isolated residual

gametes with larger red gametes obtained by lack of phosphate and nitrogen in the culture medium. She gave only a short notice on these investigations (1937), but in my book "Die Sexualitaet" (1943) I published a detailed description including the reproductions of her fine illustrations. In normal clone cultures *Haematococcus pluvialis* is isogamous and monoecious; it produces small green gametes that copulate with each other. But combinations of residual gametes hint at a clear "Zweierschema." In clone cultures without phosphate and nitrogen, big red gametes are formed that do not copulate with each other nor with other red gametes from similarly treated clone cultures. On the other hand a profuse pairing takes place with one type of the normal small green residual gametes, but just with one type only (fig. 1). Thus, a different culture method induced the formation of bigger and red gametes of only one sex which may be called female. It is impossible to demonstrate more clearly or more impressively the formation in haplomonoecious isogamous algae of two sexually different gametes, plus and minus or male and female, respectively.

Lewin, in his review, reports the earlier statements of Pascher (1931) concerning *Chlamydomonas paupera* from a natural habitat. This strain was held to be monoecious and isogamous. The released gametes were claimed to be male first and to become female afterwards. For this strain of Chlamydomonas, Moewus (in Hartmann, 1934a) proved later, with clone cultures from the same location, haplogenotypic dioecism. Further statements of Moewus on the occurrence of two different sorts of gametes in monoecious isogamous algae will not be mentioned here, since they were published only after his leaving our institute.

Lewin furthermore lays greater stress on the accounts of Pringsheim and Ondratscheck (1939), (who could not confirm the findings of Moewus on monoecious strains of Polytoma and Protosiphon with regard to the behavior of the residual gametes), than on the positive statements of Haemmerling, Rosenberg, Moewus and Lerche concerning other organisms. He attaches great weight to the objections which led Pringsheim and Ondratscheck to deny, from an evolutionary standpoint, the likelihood of the existence of two diverse sexual types in case of modificatory differentiation of sex which, according to me and my collaborators, is said to be controlled by the presence of sex substances in the nutrient medium. But this reasoning of Pringsheim and Ondratscheck is of a purely hypothetic and teleologic nature and thus cannot prove any facts. That they also failed to find two sexual types in some cases is neither remarkable nor decisive. It goes without saying that in some cases the residual gamete method will fail and it will be impossible to demonstrate the presence of sexually differentiated gametes for some monoecious isogamous algae. This would hold especially for cases in which the gametes are not released all at once but emerge only successively in the course of one day or of several days, or if a chemotactic or phototactic isolation of the residual gametes cannot be achieved. The negative results of Pringsheim and Ondratscheck might have been due to such conditions. In any case a few negative findings cannot affect the conclusive force of positive results by acknowledged reliable investigators.

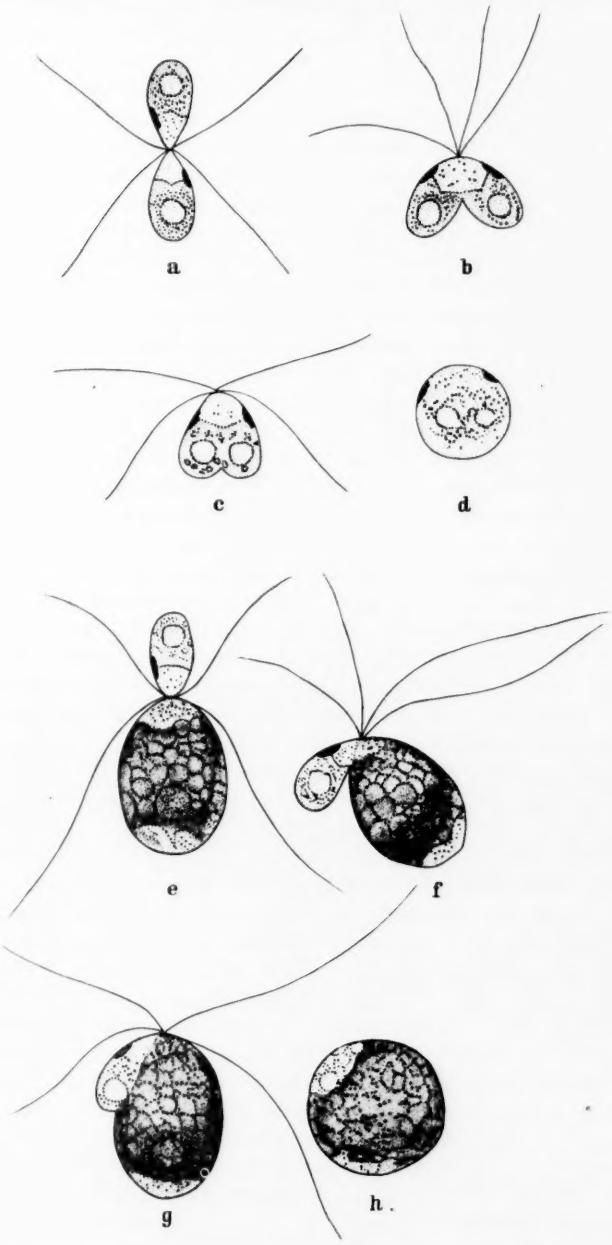

FIGURE 1. *Haematococcus pluvialis* (Phytoflagellate, Volvocale). a–d normal copulation of isogamous gametes of a ♂ clone. e–k copulation of red ♀ gamete (obtained by modification in ♂ clone cultures free from N and P) with normal small green ♂ residual gamete of a ♂ clone. After Lerche from Hartmann (1943).

In concluding this discussion of the existence of a general physiological bisexuality in isogamous algae, I wish to add some personal remarks on the reasons which led to my theoretical conception of an at least physiologic difference in genetically identical isogamous gametes and to my postulation of the occurrence of a general bisexual potency. It was not in any case what Lewin says, an "anthropomorphic concept, borrowed perhaps from Aristotle."

The notion of a general bipolar bisexuality and bisexual potency goes back to O. Buetschli (1888–1889) who presented it for the first time in his great standard work on protozoa after having recognized that his earlier "Verjüngungshypothese" (hypothesis of rejuvenescence) is unable to explain all aspects of fertilization in protozoa. While at that time the "Amphimixislehre" of Weismann and the "Verjüngungshypothese" of Buetschli were predominating, the latter's hypothesis of sexuality was left unnoticed until F. Schaudinn (1905), without having knowledge of Buetschli's conception, developed in principle the same hypothesis in a report before the German Zoological Society. Some months earlier my friend Schaudinn had talked with me about his new hypothesis. Though already at that time, on account of logical considerations as well as observations and experiments, I had come to the conviction that neither the "Amphimixislehre" nor the "Verjüngungshypothese" were satisfactory theories of fertilization, I opposed his views in long discussions just on account of the numerous cases of morphologically and physiologically isogamous algae which I had already worked on at that time. But shortly afterwards there appeared the famous publications of Blakeslee (1904, 1906) in which he demonstrated for the isogamous Mucorinea *Phycomyces blakesleeanus* the presence and the indispensability of two sexually different types, i.e. plus and minus mycelia, for fertilization. Only after inoculation of both kinds of mycelia on agar slants did there arise in the zone of their contact the isogamous gametangia which copulate.

With these findings of Blakeslee the possibility for a general bipolar bisexuality as well as for a general bisexual potency arose at one stroke. At that time it was not yet ascertained whether the bipolar bisexuality in this fungus was of genotypic origin, though that seemed most probable. Later Burgeff (1914) finally established this fact. Phycomyces remained for a long time the only case of such a genotypic dioecism. Only after 1919 could Kniep (1919) and his successors demonstrate the unexpected frequency of haplogenotypic dioecism in other isogamous fungi (Uredineae and Hymenomycetes), while later Kniep's students established the same fact for fresh water algae and I, with my collaborators, did the same for marine algae. From the year 1925 onward it was proved that in isogamous and anisogamous algae a strict differentiation of sex may occur not only as a genotypic difference but also as a modification (phaenotypic dioecism). At the same time slight cytological differences of gametes in seemingly isogamous algae and protozoa, or a diverse behavior during fertilization was repeatedly observed. The extreme multiplicity of the various genetic and cytologic

findings and the manifold interlacing ways for their demonstration naturally cannot be described in this survey.

Protozoa. I confine myself to a short report of some recent cytological findings by Cleveland (1953) in his investigations of the hypermastigote flagellates, parasitic in the wood-feeding roach *Cryptocercus punctulatus*. These studies prove a bipolar bisexuality of the fusing nuclei even in cases of extremely autogamous fertilization. Thus Barbulanympha is an anisogamous genus of flagellates the species of which differ with respect to the occurrence or non-occurrence of cell division following the nuclear division that initiates sexual differentiation. For instance, in *Barbulanympha ufalula*, this cell division usually occurs and so leads to normal complete gametogenesis, while in *Barbulanympha wenyoni* it usually fails to occur so that gametogenesis remains incomplete. According to Cleveland the accompanying nuclear division in all species differs from asexual mitosis. In the derived male nuclei the telophase is completed more quickly. They can therefore be identified at an early stage by the denser coiling of the chromosomes and accordingly smaller size as compared with the female nuclei (fig. 2). In contrast with Trichonympha (see page 17), the final gametes differ only in size, the androgametes being smaller than the gynogametes. If, as is usually the case in *Barbulanympha wenyoni*, gametogenesis is not completed, an autogamous fusion of the pronuclei takes place. In other words, we can observe in just one genus the transition from anisogamous gametogamy to clear autogamy and moreover a sexual differentiation of the fusing pronuclei in extreme autogamy. Furthermore, as is the case in all hypermastigote flagellates, only the female centriole is preserved while that of the male pronucleus is eliminated. This holds for normal gametogenesis (*Barbulanympha ufalula*) as well as for extreme autogamy (*Barbulanympha wenyoni*).

RELATIVE SEXUALITY IN ALGAE, FUNGI AND PROTOZOA

Algae. The occurrence of relative sexuality is mentioned by Lewin only incidentally and insufficiently. He states only that Moewus reported for several algae (e.g. Chlamydomonas, Polytoma and Protosiphon) obtaining viable zygotes by mating strong gametes with weak gametes of the same genetic sex. Extensive physiological and genetic investigations for many such cases are said to have been described by Moewus, and these were reviewed by Moewus (1941, 1951), Chodat (1941), Smith (1951) and others. "Relative sexuality has apparently not been established in any unicellular alga investigated by other workers."

Foerster and Wiese (unpublished) could not confirm Moewus on the occurrence of relative sexuality in strong and weak mutants of *Chlamydomonas eugametos* (put at our disposal by Moewus). I convinced myself of the correctness of their observations. Further experiments on relative sexuality with these mutants were not continued, for our main purpose was to clarify the chemical nature and biological efficiency of the gamones in *Chlamydomonas eugametos* (see below, page 000), on which Moewus had reported.

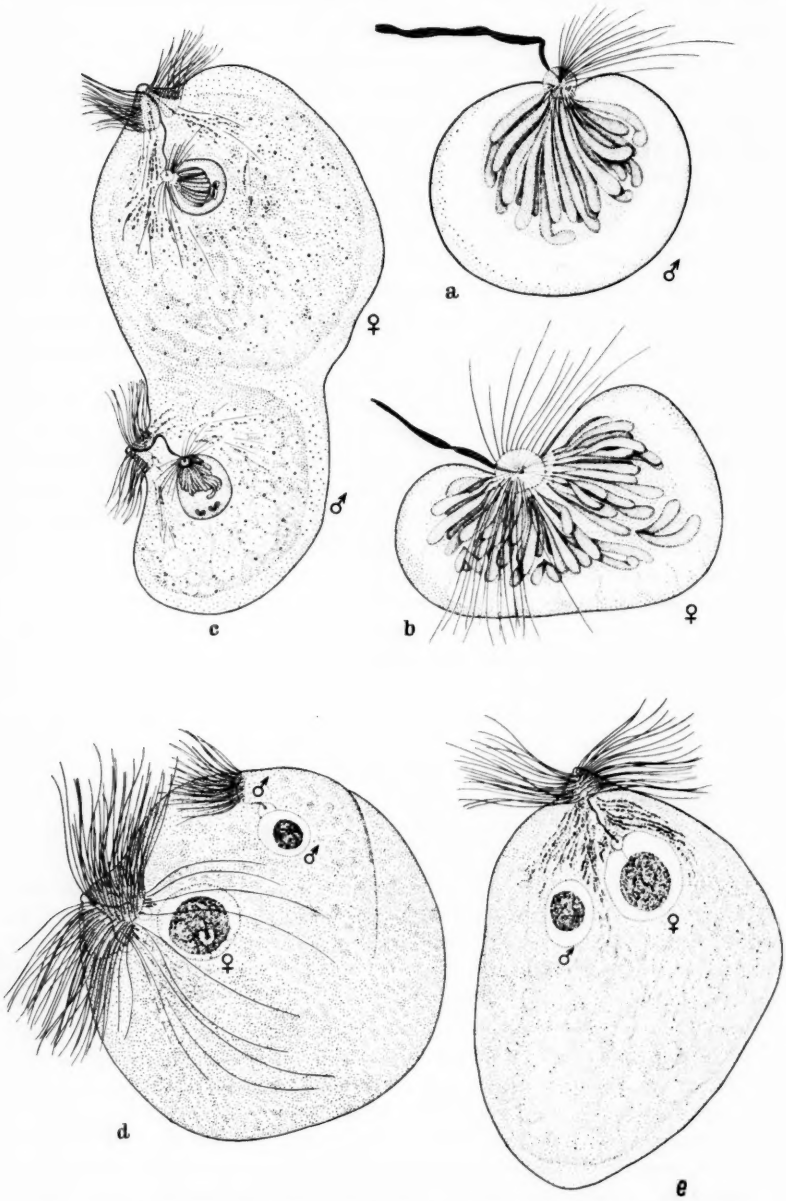

FIGURE 2. Farbulanympha (hypermastigote flagellate). a) and b) ♂ and ♀ daughter-nucleus in telophase after cytokinesis of a gametocyte containing a haploid nucleus, x 1400; c) beginning cell division in the smaller ♂ and larger ♀ gamete; d) anisogamous copulation of a ♂ and a ♀ gamete after cytoplasmic fusion, ♂ centriole and flagella in process of elimination; e) zygote shortly before caryogamy, ♂ extranuclear organelles disintegrated, x 400. After Cleveland (1953).

Nevertheless I still believe that his described cases of relative sexuality in crosses of *Chlamydomonas paradoxa* with *Chl. pseudoparadoxa* are correct. But being so far unconfirmed, they are left out of consideration here.

The indication for the occurrence of relative sexuality in algae with isogamous fertilization, in forms with modificatory or genotypic dioecism, is in my opinion one of the most important and decisive points for the validity of the general sex hypothesis of fertilization. For this reason I wish to furnish a more detailed proof of it.

In 1909, following a review of all available accounts of automictic fertilization in protists and thallophytes, I advanced the thesis that, because of certain findings, a general theory of sexuality and fertilization can prove correct only if the assumed bisexuality could be confirmed as relative, not as absolute. From this hypothesis, strictly logical deduction led to the conclusion that it should be possible experimentally to demonstrate cases in which gametes A act as males in relation to gametes B, while they act as females in relation to gametes C. The first proof of such a relative sexuality I found in 1914 together with Dr. Huth at the Zoological Station of Lunz am See in a Spirogyra of undetermined species (see Hartmann, 1923). When transferred to rainwater a profuse pairing took place. Besides normal ladder-shaped copulations, i.e. of strictly dioecious type, we found conjugations between three different filaments of the algae, of which two were either

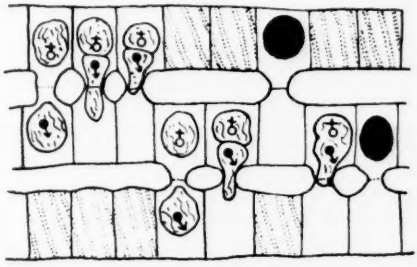

FIGURE 3. Spirogyra spec. (Chlorophycea). Copulation of three filaments, half schematic. A = proper ♀, B = proper ♂, C is ♂ with regard to A, ♀ with regard to B, relative sexuality. After Hartmann (1923).

proper male or female while the third one copulated with the gametes of the female as well as with the male filament, exactly as I had postulated in 1909 and tried to confirm by experiments. Figure 3 gives a half schematic reproduction of the finding. Unfortunately it was not possible to cultivate this strain. On account of the outbreak of the war we had to return to Berlin-Dahlem and the material perished. A thorough analysis of the subject and further experimental tests became thus impracticable. Since at that time nothing was yet known about dioecious haplonts with non-hereditary modificatory sex determination, we assumed that we had found a case of haplogenotypic dioecism with relative sexuality. Later Czurda (1925) established for *Spirogyra weberi* that its strict dioecism can be accomplished only by modification (phaenotypically).

After the war experiments on relative sexuality in algae could not be resumed until the spring of 1924 at Naples. Besides numerous combinations between normal male and female gametes in strictly dioecious *Bryopsis mucosa* as well as between two female or two male types of different plants, respectively, I observed with Belař (see Hartmann, 1929a) in a combination of gametes from two different female plants several normal fertilizations which are copied here from drawings by Belař (fig. 4). We were not able to raise cultures from these zygotes at that time. Further experiments on Bryopsis were not undertaken because during the following spring we found in the

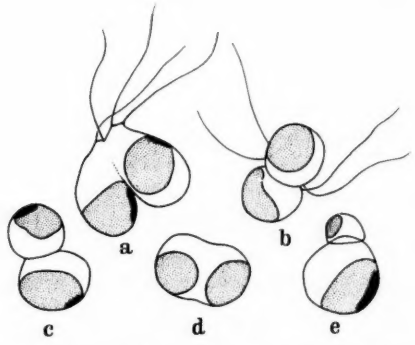

FIGURE 4. *Bryopsis plumosa* (Chlorophycea). Copulation between ♀ gametes from different plants. After Hartmann (1929a).

Phaeophycean, *Ectocarpus siliculosus*, an organism much better suited for the demonstration of relative sexuality.

In the autumn of the same year I made combinations with the isogamous gametes of *Dasycladus clavaeformis* which proved strictly dioecious during the time of my tests. Among a great number of normal combinations, following strictly a "Zweierschema," I found one case of relative sexuality. In the autumn of 1925 the sexual conditions in this alga were thoroughly investigated by my collaborator at that time, Jollos (1926), who demonstrated the occurrence of relative sexuality in this species. His results were discussed in detail in the book of Kniep (1928) and again by myself (1943) so that I can refer to these reports.

The best object to demonstrate the occurrence of relative sexuality in algae proved to be the multicellular Phaeophycean, *Ectocarpus siliculosus*, from Naples (not from other habitats). For this alga Berthold (1881) and later Oltmanns (1899) had already demonstrated that only gametes originating from different plants can copulate. As these two botanists had established, the morphologically isogamous gametes behave differently when brought together; while one (the female) type settles down and loses its flagella, the other (the male) type aggregates around the fixed females by chemotaxis. This is followed by fusion of one male gamete with a female one (fig. 5).

In 1925 and 1926 (in 1925 supported by Jollos) we gathered the gametes of 155 plants of Ectocarpus and made 1400 combinations between male and female, female and female, and male and male gametes which confirmed and completed the statements of Berthold and Oltmanns. We could ascertain that in combinations of male and female types of gametes a violent clumping took place, sometimes in a few seconds, while in other cases we observed a slower clumping with less groups or, still later, a very weak clumping. This shows that there exist strong females, rather strong females and weak females and conversely strong male, medium strong male and weak male types of gametes. In Ectocarpus the same plants may release their

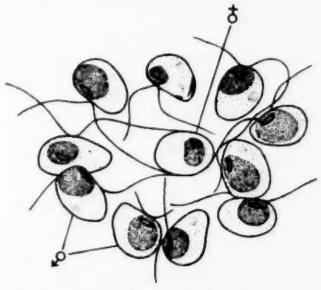

FIGURE 5. *Ectocarpus siliculosus* (Phaeophycea). Copulation of gametes, seen from above. Drawn from stained material. After Hartmann (1937).

gametes in the course of several days up to 6 or 8 times; and in all ensuing tests the sexual strength of each plant proved to be always of the same degree. On combination of strong and weak gametes from plants of the same sex we observed relative sexuality in 38 cases. Often one type was shown to be strong, another one of the same sex to be weak (table 2). The objections by Czurda and Mainx against this interpretation of the experiments—Czurda had assumed hidden monoecism—I had considered myself and taken into account in 1925 and in full particular in 1926; but in all cases I could establish strict dioecism. A few cases which seemed to be exceptions to the rule could be explained by interlacing of two plants or otherwise (compare Hartmann, 1934b). My collaborators Haemmerling, Föyn and Bauer have examined these relations in later years with the same results. Furthermore (Hartmann, 1937) it could be ascertained by marking one or both types by vital staining that always the female gametes settle in the middle of a clumping group while the males keep their motility. After so many detailed trials there can be no further doubt about the occurrence of relative sexuality in this morphologically isogamous but physiologically anisogamous Phaeophycean. Should someone not yet be convinced of the right interpretation of the Ectocarpus tests, I recommend a thorough examination of the cited publications (1925 and especially 1934b and 1937) in which the possible sources of error are discussed in detail and the interpretation of Czurda was shown to be incorrect. After my paper of 1934b neither Czurda nor Mainx has repeated his doubts or objections concerning the experiments with Ectocarpus.

TABLE 2

ECTOCARPUS SILICULOSUS (PHAEOPHYCEA). COMBINATIONS OF GAMETES OF 6 PLANTS. NO. 33, 35, 38 AND 40 = ♂♂; NO. 31 AND 32 = ♀♀. THE NUMBERS 1, 2 AND 3 INDICATE THE DIFFERENT INTENSITIES OF COPULATION. THE WEAK ♂ NO. 35 GIVES A POSITIVE REACTION WITH THE STRONG ♂♂ NO. 33 AND NO. 38, RESPECTIVELY. AFTER HARTMANN, 1925.

	♂ 33	♂ 35	♂ 38	♂ 40	♀ 31	♀ 32
♂ 33	-	1	-	-	3	3
♂ 35	1	-	1*	-	3	2
♂ 38	-	1*	-	-	3	3
♂ 40	-	-	-	-	3	2
♀ 31	3	2	3	3	-	-
♀ 32	3	2	3	2	-	-

	♀ 34	♀ 37	♀ 39	♀ 41	♀ 43
♂ 33	3	2	3	2	3
♂ 35	1	2	1		2

* 3 groups

By (parthenogenetic) culture of isolated gametes of Ectocarpus, Haemmerling (see Hartmann, 1934b) established that male gametes can give rise to either male or female plants. The strict dioecism of *Ectocarpus siliculosus* from Naples thus proves conditioned by modification (phaenotypically), as likewise ascertained for other algae in the meantime.

Now it was of importance to furnish proof of relative sexuality in algae with haplogenotypic sexuality. Besides the cases described by Moewus (that became doubtful, see above, page 000) such a case came to observation only once in *Enteromorpha ramulosa*. Table 3 shows that the gametes of plant number 2 (minus) gave weak clumping and copulation with those of plant number 11 (minus) (Hartmann, 1929b). Unfortunately it was not possi-

ble at that time to keep cultures of this alga, so that a further experimental genetic analysis could not be carried out.

Fungi. I confine myself to a short description of conclusive findings of relative sexuality in Ascomycetes and Basidiomycetes (especially Hymenomycetes). I wish to emphasize that at an early date Blakeslee and his collaborators had already described in Mucorinae a strong difference of valence in infertile crosses between various dioecious as well as between monoecious and dioecious species and made probable the occurrence of relative sexuality.

TABLE 3

ENTEROMORPHA RAMULOSA (CHLOROPHYCEA). COMBINATIONS OF 4 PLUS GAMETES AND 4 MINUS GAMETES. 3 AND 1 INDICATE THE INTENSITY OF THE CLUMPING REACTION. ! IS A CASE OF RELATIVE SEXUALITY. AFTER HARTMANN, 1929b.

		+				−			
		1	5	8	10	2	6	9	11
+	1	0	0	0	0	3	3	3	3
	5	0	0	0	0	3	3	3	3
	8	0	0	0	0	3	3	3	
	10	0	0	0	0		3	3	3
−	2	3	3	3		0	0	0	1!
	6	3	3	3	3	0	0	0	0
	9	3	3	3	3	0	0	0	0
	11	3	3		3	1!	0	0	0

For Ascomycetes Huettig (1935) first demonstrated in *Glomerella lycopersica* the occurrence of forms with different sex valence. He employed hermaphrodites and genetic males obtained from a hermaphroditic stock by natural or experimentally induced mutations. Thus the conditions for relative sexuality are given in this instance. Furthermore the various sexual reactions of this Ascomycete, on pairing of hermaphrodites alone or hermaphrodites with males of different valence, revealed relations that can be interpreted as relative sexuality.

An incontestable statement came from Greis (1941) who worked on mutants of *Sordaria fimicola*. By X-radiation of a hermaphroditic initial form he got strong, normal and weak females as well as strong, normal and weak males. When he combined strong female mutants F^3 with weak female mutants F^1 he obtained in several cases normal fruiting bodies. Since the female sex is to be recognized plainly by formation of ascogonia it is beyond doubt that in

TABLE 4

SORDARIA FIMICOLA (ASCOMYCETE). O = NO FRUIT-BODIES, Z = FERTILE FRUIT-BODIES, x = STERILE FRUIT-BODIES, x A CASE OF WEAK FERTILITY. AFTER GREIS, 1941.

(8112 × 8747)	1	2	3	4	5	6	7	8
921 ♀	x	x	0	0	0	0	x	x
2267 ♂	Z	Z	Z	Z	Z	Z	Z	Z

this case mycelia of the same sex came to fructification (table 4). This result he furthermore confirmed by analysis of gones.

For Hymenomycetes Quintanilha (1939) has shown by a very careful genetic analysis of the so-called Buller phenomenon in mutants of *Coprinus fimetarius* that the A-factors, which according to later investigations of Greis are to be looked upon as the essential sex factors, possess different strengths in the mutual attraction of nuclei in haplomycelia with respect to the weaker type of nuclei in diplomycelia. Quintanilha thus came to the important establishment of a differing valence in the multiple series of A-factors as expressed by the following schema:

$$\underset{10}{a} \text{ -------- } \underset{20}{A} \text{ -------- } \underset{40}{A_1} \text{ -------- } \underset{50}{a_1}$$

In this connection he hints at the fundamental similarity between the differing valences of A-factors and the occurrence of relative sexuality in algae.

TABLE 5

SOLENIA ANOMALA (BASIDOMYCETE). COPULATION IN ♀ STRAINS OF DIFFERENT VALENCE, SHOWING RELATIVE SEXUALITY. − = NO COPULATIONS; + = FRUIT-BODIES; S = COPULATIONS, BUT NO MATURE FRUIT-BODIES. AFTER GREIS, 1942.

	F^2 W_2	F^2 W_4	F^2 W_8	F^2 W_{10}	F^2 W_{14}	F^4 W_{16}	F^3 W_{18}	F^1 W_{21}
W_2 F^2	—	—	—	—	—	S	—	—
W_4 F^2	—	—	—	—	—	S	—	—
W_8 F^2	—	—	—	—	—	S	—	—
W_{10} F^2	—	—	—	—	—	S	—	—
W_{14} F^2	—	—	—	—	—	S	—	—
W_{16} F^4	S	S	S	S	S	—	—	+
W_{18} F^3	—	—	—	—	—	—	—	S
W_{21} F^1	—	—	—	—	—	+	S	—

The reasons which induced him to refuse nevertheless a homology of A-factors and real sex factors have been removed by the subsequently published account of Greis (1942) which shows for the Hymenomycete *Solenia anomala* that A-factors are true sex factors.

In this fungus, *Solenia anomala*, Greis (1942) furthermore observed among the female stocks some of a hereditarily different valence, viz. four females of different strength (F^1, F^2, F^3 and F^4) which after combination produced some cases of relative sexuality. While F^1 and F^3 as well as F^2 and F^4 are able to copulate normally with each other but do not form fruiting bodies, $F^1 \times F^4$ copulate and moreover yield normal fruit-bodies (table 5). These clear results allow the conclusion that the described findings of Quintanilha are also to be regarded as cases of relative sexuality. By this short review on anisogamous Ascomycetes and isogamous Hymenomycetes with haplogenotypic determination of sex, the occurrence of relative sexuality in these fungi is demonstrated beyond doubt.

Protozoa. Cleveland (1949), in his account of the fertilization of the hypermastigote flagellate Trichonympha, described the most impressive case of relative sexuality that has yet come to our knowledge. In his publication he called the gametes with abnormal behavior and weaker sexual differentiation gynandromorphic, but after my review (Hartmann, 1954) of his many interesting results on the fertilization of hypermastigote flagellates he accepted my interpretation of the observations. The gametes of this haploid flagellate—which are distinctly characterized as male and female by their cytologic structure and by their behavior during fertilization—arise within a cyst by a single division of one cell (gametocyst). Already in the prophase and early metaphase a darker staining intensity of the chromatids of the later sperm nucleus, when compared with that of the later egg nucleus, is to be observed as the first indication for a sexual differentiation of the two later gametes. The cytoplasmic differentiations, which as a rule occur only after excystation of the gametes, are most impressive: while the granules in the male gametes remain scattered in the cytoplasm, they gather in the posterior end of the egg and congregate to a compact ring of fertilization granules around a lucid mass of cytoplasm, serving as fertilization cone (fig. 6Ib). At this point the male gamete enters the egg like a sperm and, while the cytoplasm is taken in, the male extranuclear organelles degenerate and the darker stained male gamete fuses with the female. The fertilization process of Trichonympha thus proves to be extremely anisogamous regarding the cytologic structure and the conduct of the gametes, while the varying sizes of the latter are without sexual significance.

Fertilization in Trichonympha, as in all hypermastigote flagellates occurring in the blattid *Cryptocercus punctulatus*, takes place epidemically in close correlation with the molting of the host. Besides numerous normal copulations there occur fusions of two female or two male gametes of cytologically varying differentiation (valence). Figure 6IIa shows pairing of two gynogametes of which the upper one is of normal female structure with a compact ring of fertilization granules at the posterior end, while the lower

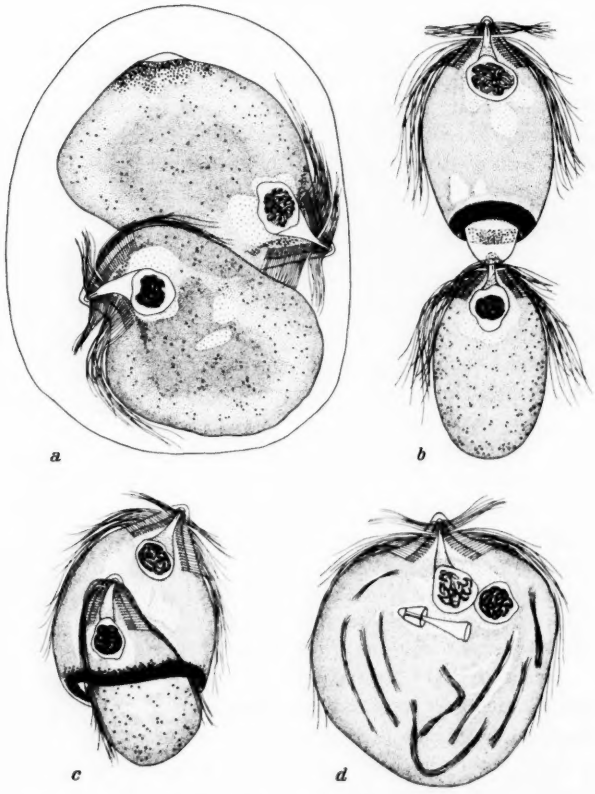

FIGURE 6. Trichonympha (hypermastigote flagellate). Ia–d fertilization. a) ♂ and ♀ gamete before leaving the gametocyst. The upper ♀ gamete shows the very exceptional early development before excystation of the ring of fertilization granules and the fertilization cone. b) beginning copulation of the completely differentiated ♂ and ♀ gametes. c) ♂ gamete entering further into the ♀. d) fusion of cytoplasm completed, ♂ extranuclear organelles in disintegration, first stage of caryogamy.

one is entering sperm-like and possesses only weak female properties: the granules remain partly scattered in the cytoplasm, as is the rule with androgametes, the ring of fertilization granules at the posterior end is not perfect and the fertilization cone is missing. In the case demonstrated in Figure 6IIb, the female character of the gynogamete, behaving like a sperm, is even more explicit. An example of special interest is reproduced in figure 6IIc, where three gynogametes of relative sexuality with more or less female characteristics are in copulation. Here the lowest one shows prevailing male properties in that the accumulation of the granules at the posterior end has just begun. Cleveland has published in his paper mostly cases of female relative sexuality; but in addition mating may occur between two andro-

gametes with different degrees of the male characteristics as shown by figure 6IId.

The natural occurrence of relative sexuality in a protozoon, the gametes of which are extremely anisogamous in their cellular structure as well as in their conduct, and its simple microscopic establishment is most astonishing. In Ectocarpus it was necessary to make more than a thousand gamete combinations to refute all possible objections and to secure the result (Hartmann, 1925, 1934b, and 1937). Here it is sufficient to fix and stain some preparations and take a look into the microscope to be convinced of the facts! Moreover they can be studied on living material. Even the boldest imagination would have scarcely dared to dream of such an anisogamous

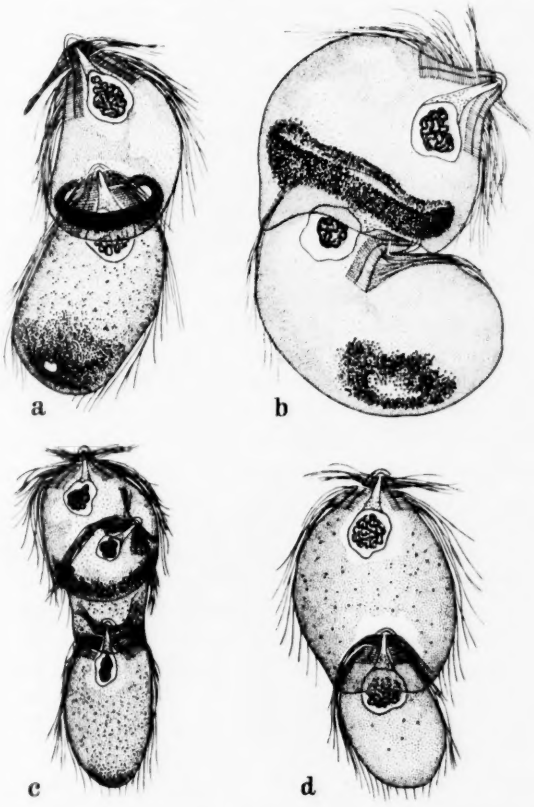

FIGURE 6. IIa–d relative Sexuality. a) weak ♀ gamete entering a normal gynogamete. b) gynogamete of even stronger ♀ differentiation behaving like a ♂. c) fusion of three ♀ gametes, the second one of weaker ♀ differentiation than the upper one, the third one with even less ♀ characteristics. d) attempted fusion of two ♂ gametes, the entering lower one with less granules. x 420. After Cleveland (1949).

fertilization and at the same time the different degrees of sexuality (valence) as realized in the case of Trichonympha. The very degree of female tendency in the gynogametes of a relative sex can be estimated by eye. Though today the occurrence of relative sexuality may be called experimentally proved for Ectocarpus and other algae and fungi (see above), such an impressive microscopically visible proof as furnished by the observations of Cleveland on Trichonympha will have still more convincing power for quite a number of biologists since many of them (not to say most of them) are inclined to believe only their eyes.

GAMONES

Algae. If gametes of the same sex of *Ectocarpus siliculosus* are brought together nothing happens, even if hundreds or thousands of gametes are whirling about. If they cling to each other occasionally, they will soon separate again. But in combinations of male and female morphologically isogamous gametes with full reaction capacity the whole Boveri-dish shows suddenly in a few seconds a heavy clumping. In the middle of each such cluster is placed a female gamete without flagellum, surrounded by swarming males, until finally one male gamete fuses with the immobile female. With this observation in 1925 we grasped at once the conception that this strange behavior could be explained only if the androgametes secreted a substance that induces the gynogametes to throw off their flagella and become fixed; and if the females produced some other substance that calls forth the approach of the males by chemotaxis or thigmotaxis. Attempts to produce a specific reaction in the opposite sex by means of filtrates of isolated female and male gametes failed in Ectocarpus in the years 1925 and 1926. But my collaborator Jollos could establish in the autumn of 1925 in *Dasycladus clavaeformis* such a specific efficiency of filtrates from plus or minus gametes. For this demonstration he used minus gametes with lessened reaction capacity as shown by their failure to yield further clumping and pairing when mixed with gametes of the opposite sex. Such unreactive minus gametes, when placed in filtrates from minus gametes of undiminished reaction capacity, at once yielded again profuse formation of clusters and pairs after being mixed with plus gametes (This is Lewin's case 2 in his account of the different actions of sex substances). In tests on filtrate efficiency, Jollos further found that the filtrates of gametes with strong reaction capacity may change the response of weakly reacting gametes of the same sex, namely, that the latter become capable of copulation with strong gametes of the opposite sex or, in other words, that weakly reacting gametes were unable to copulate with gametes of the opposite sex unless they were subjected first to filtrates of strong gametes of the same sex as that of the weak gametes. The substances in question were called later by Kuhn and Moewus termones (case 4 of the different actions of sex substances according to Lewin). With these experiments Jollos had already in 1926 ascertained substances of type 2 (gamones) and type 4 (termones). At first Jollos regarded the substances ascertained by himself merely as the cause

of copulation. In contrast with me he did not attach to them a special value as sexual substances. But later results concerning sexuality in algae and fungi demonstrated that such a distinction between substances inducing copulation and special sex substances is without relevance in this form. I refrain from going into detail on sex-determining substances.

A further proof of gamones (substances of type 3 according to Lewin) was furnished by Geitler (1931) for *Tetraspora lubrica*, a strain with haplogenotypic determination of sex. He found that the gametes of one genetic sex could be induced to give a clumping reaction (autoagglutination) by the addition of concentrated extracts practically free from cells, gained from suspensions of the opposite sex. By this test for the first time the case 3 of Lewin was established. Similar results were reported by Moewus (1933) regarding filtrates of *Chlamydomonas eugametos*, contaminated with bacteria, and residual gametes of Stephanosphaera and Protosiphon.

Before entering into particulars on the accounts of Moewus which recently were confirmed in principle by Foerster and Wiese, though they had to be revised in some essential points, further positive findings of gamones in unicellular and multicellular algae will be reported. Thus Lerche (1937) could account for sex specific gamones in the haplogenotypic dioecious *Dunaliella salina*. Instead of extracts (as in the tests of Geitler) she used centrifugates from gametes of one sex with which she induced clumping reaction in the gametes of the other sex (autoagglutination). She found that by filtration through a membrane with 0.75 μ pores the active agent was removed, and she suggested that larger particles rather than a solute were responsible for the effect. The accounts of Geitler and Lerche are well-founded by their protocols, and in the case of Lerche furthermore

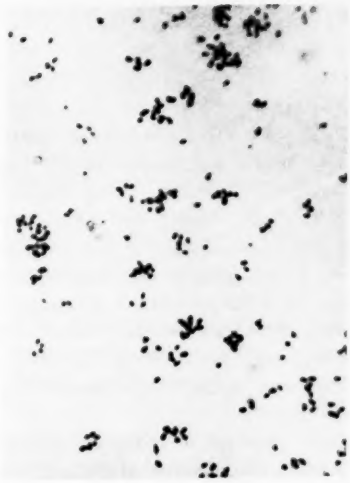

FIGURE 7. *Dunaliella salina* (Chlorophycea). Gamone evidence by clumping reaction of a + type of gametes after application of centrifugates of a − type. Autoagglutination. After Lerche (1937).

by a photocopy of a clumping reaction induced by filtrates of gametes of the opposite sex (fig. 7).

In *Ectocarpus siliculosus* the occurrence and the effect of gamones was established finally in the spring of 1936 (Hartmann, 1937). In that spring (though only after the end of April) Ectocarpus proved to furnish fine material. All of the 64 tested plants, with the exception of only four specimens, released an abundance of gametes (most of the plants several times, that is, up to seven times in the course of some days), and all of them were dioecious. This strict dioecism showed up usually between 9 and 10 a.m. before the starting of combinations since the female gametes exhibit less motility. They often lost their flagella and had already settled down during the forenoon. For this reason some of the female types, which in the early morning had paired with heavy clumping reaction, by about noon gave no more clusters nor copulations with their still fit male partners. But with the same males a renewed good reaction could be induced by the addition of filtrates from three to four female types that had kept motility (case 2 of the different actions of sex substances according to Lewin). In a similar way the effect of male sex substances (gamones) could be demonstrated.

Lewin's statement: "apart from this uncertain case" (with reference to an account of Schulze, 1927, unmentioned in this report) "sex substances have apparently been reported in unicellular algae only by Moewus," does not prove correct according to the investigations just described. For unicellular algae the occurrence of gamones and their activity had been established beyond doubt by Jollos (1926) in Dasycladus (case 2 of Lewin), by Geitler (1931) and Lerche (1937) in Tetraspora and *Dunaliella salina* (case 3 of Lewin, and by Hartmann (1937) for the multicellular Phaeophycean *Ectocarpus siliculosus* (case 2 of Lewin). At the same time the occurrence of termones had already been ascertained by Jollos (1926) in Dasycladus (case 4 of Lewin).

Following this historical review of the evidence I shall discuss in particular the reports of Moewus on the presence of gamones in *Chlamydomonas eugametos*. In the last year or two my collaborators Foerster and Wiese (1954 a and b) have submitted these questions with regard to *Chlamydomonas eugametos* to a thorough examination. They could to some extent confirm the account of Moewus (1933), though not with regard to some rather vital points. One of these concerns the statement of Moewus that the clumping of gametes of one sex by filtrates of the opposite sex (autoagglutination, case 3 of Lewin) can be induced only if bacteria are present or applied simultaneously. Divergently Foerster and Wiese obtained the same result with filtrates absolutely free from bacteria (fig. 8). This item is of minor importance, since Moewus himself mentioned the desirability of its reexamination.

Of essentially greater importance is the second deviation from Moewus' report. This concerns the dependence of gamone formation on exposure to light, a relation which later assumed great importance in Moewus' claim for the carotinoid nature of the gamones. In the first place, Foerster and Wiese

(1954 a and b) established that the gametes are already completely motile in the dark, while Moewus had stated that they become agile only after exposure to light or to the carotinoid crocetin, formed in the light. Secondly, Foerster and Wiese found that the female gametes have perfect capability of reaction even without illumination. The female gamone is already released in the dark and accordingly the gynogametes can at once start clumping and copulation with males made capable of reaction by light; while the male gamone is released by the androgametes to the culture medium only after exposure to light. These exact results of Foerster and Wiese show that by performing the experiments indicated by Moewus it is quite impossible to demonstrate the said gamones or compounds of cis- and trans-crocetin-dimethylester and their dependence upon light. Furthermore they (unpublished) could prove by spectroscopic methods in a 28-liter-suspension of

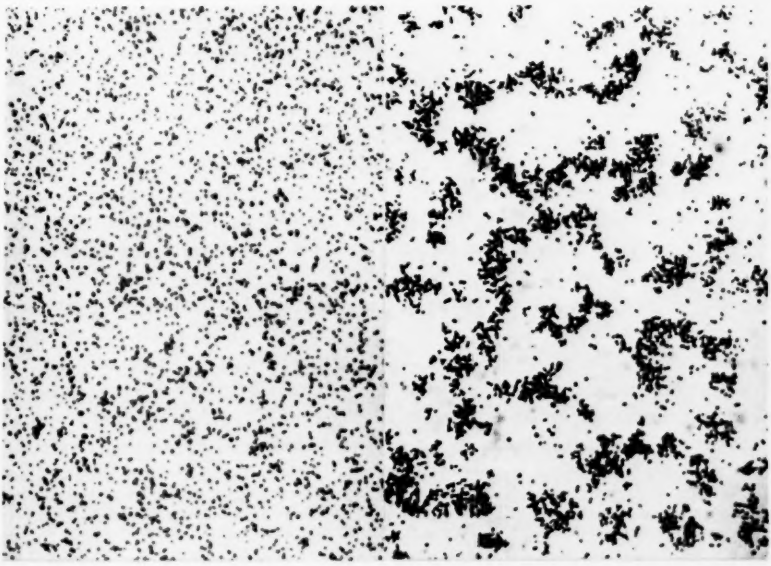

FIGURE 8. *Chlamydomonas eugametos* (Chlorophycea). Gamone evidence by clumping reaction of a + type of gametes following application of filtrates of a − type. a) ♂ cells. b) ♂ cells after addition of ♀ filtrate. Autoagglutination. After investigations of Foerster and Wiese (unpublished).

Chlamydomonas eugametos the complete absence of crocin. These results are thus in obvious contrast with the record on chemical substances found by Kuhn and his collaborators in filtrates from cultures furnished by Moewus. In this connection I wish to add only that Foerster and Wiese could prove without a doubt by chemical investigations, especially by paper chromatography, that the gamones of *Chlamydomonas eugametos* in filtrates consist of high molecular weight proteins with a sugar component (so-called glycoproteids). These are examples of the same group of chemical substances

accounted for as agglutinating gynogamon II in the sea-urchin (Vasseur, 1952 and Wallenfels, 1955) as well as in the trout (Hartmann, Medem, Kuhn and Bielig, 1947).

Also in *Chlamydomonas reinhardi* Foerster and Wiese (1955) could establish in the same reliable way the clumping by filtrates of the opposite sex. For all further details on the recent results of the cited workers I refer to their publications or those in press. In these investigations, most accounts, especially of American authors, that were in disagreement with the statements of Moewus find an explanation.

Fungi. Burgeff (1914) established for fungi, by his experiments with the mucorale *Mucor mucedo*, a gamone-like effect of chemomorphotically efficient substances. The minus mycelium, separated by a collodion wall from the adjacent plus mycelium, not only induced the formation of zygophores

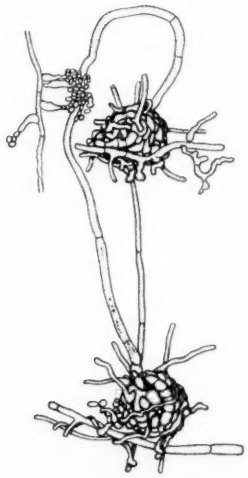

FIGURE 9. Bombardia (Ascomycete). Characteristic growth of two trichogynes to approach an accumulation of spermatia (chemotropical growth). After Zickler (1953).

through this membrane but also induced the zygophores to establish contact with the wall by growing telomorphotically backwards, instead of straight upwards. The mutual approach by growth of the hyphae and their fusion at the rim of the wall is effected obviously by substances which may justly be called gamones. The inauguration of zygophores is caused presumably by other substances like those found by Raper in *Achlya amphisexualis*.

For this Saprolegniacean Raper (1939 and 1940) has shown in a series of careful experiments that four hormone-like substances (A, B, C and D) induce the formation and the joining of antheridia and oogonia, of which A and B can be regarded as termones. The hormone C, released by the immature oogonia, causes the branches of the antheridia to grow chemotropically toward the oogonia, to be cut off and to unite with the oogonia. The op-

posite effect is produced by a substance emitted by the antheridia which calls forth the discharge of the oogonia (hormone D). It goes without saying that the hormone C can be called a gynogamone and its antipode D an androgamone.

In a third fungus, the ascomycete Bombardia, Zickler has proved the activity of an androgamone, with which I have already dealt in my book on sexuality (1943). For the final work of this investigator who perished in the war, the reader is referred to his last publication (Zickler, 1953). The male spermatia of Bombardia secrete a substance that induces the trichogynes, which grow straight in monospore cultures, suddenly to assume a meandering growth, as in combinations of male and female mycelia, and to approach the spermatia even from a considerable distance (fig. 9). Trichogynes of a female mycelium show the same behavior if capillaries, loosely fixed to the agar dishes, contain a suspension of spermatia or an extract of spermatia obtained by heating. Finally the application of agar cubes soaked with a filtered suspension of spermatia brought about an equal reaction by the trichogynes, but not by the other hyphae.

SUMMARY AND CONCLUSIONS

The following conclusions can be drawn from our critical review of the present results regarding the sexuality of algae, fungi and protozoa:

1. By the residual gamete method of Haemmerling bipolar bisexuality is absolutely demonstrated for the haplomonoecious isogamous algae Acetabularia (Haemmerling, 1934), *Stephanosphaera pluvialis*, *Protosiphon botryoides* (Moewus, 1933) and *Haematococcus pluvialis* (Lerche, 1937) as well as for the multicellular alga Hydrodictyon (Rosenberg, communicated by Hartmann, 1932). For these species, the investigations also demonstrate bisexual potency (bisexual reaction norm of Goldschmidt). Furthermore, recent cytological investigations by Cleveland on hypermastigote flagellates proved for all forms, including the seemingly isogamous and autogamous cases, a bipolar bisexuality and a bisexual potency. In the related anisogamous genera and species the exhaustive examinations by Cleveland have shown that during copulation the male extranuclear organelles are discharged while only the female ones remain intact. The apparently isogamous and autogamous forms behave in the same way, so that here too a male and a female partner can be discerned; in other words, a bipolar bisexuality is manifested. Thus we have for the first time a proof of bipolar bisexuality and bisexual potency regarding autogamous fertilization.

2. In dioecious algae in which the determination of sex is controlled either genetically (haplogenetically) or by modification (haplophaenotypically), a general bipolar bisexuality and bisexual potency is established by the occurrence of relative sexuality. Excluding single cases lacking close analysis (*Bryopsis mucosa*, *Spirogyra spec.* and *Enteromorpha ramulosa* following Hartmann), decisive evidence was secured by thorough investigations on the morphologically isogamous Phaeophycean *Ectocarpus siliculosus* (Hartmann, 1925, 1934b, 1937). Relative sexuality and thereby bisexual

potency and relative differences in valence of the sexually differentiated gametes were confirmed for anisogamous ascomycetes by Huettig (1934) in Glomerella and by Greis (1941) in *Sordaria fimicola*. The same was proved by Greis through careful genetic analysis of the isogamous hymenomycete *Solenia anomala*. These investigations render irrelevant the objections formerly raised against the interpretation of relative sexuality for the results of Quintanilha on Buller's phenomenon in *Coprinus fimetarius*. The elucidation of these sexual problems, which is most significant for the understanding of sexuality and for their explanation as relative sexuality, is now beyond doubt. All these points, including those from algae as cited above, are absolutely independent of a confirmation of the questioned accounts by Moewus. This refers in particular to the impressive, purely cytological demonstrations on the occurrence of relative sexuality in Trichonympha by Cleveland.

3. The same applies to the establishment of sex-specific (plus and minus or male and female) gamones that render possible the fusing of gametes. Only with the concept of gamones do we acquire a physiological understanding of copulation, not only with regard to isogamous but also with regard to anisogamous and oogamous gametes. This proof was secured by Jollos (1926) in Dasycladus, by Geitler (1931) in Tetraspora, by Lerche (1937) in Dunaliella and by Hartmann in *Ectocarpus siliculosus*. As shown above, the same refers in principle to *Chlamydomonas eugametos*, the object of Moewus, though some of his reports, especially the biochemical parts, did not prove correct. Gamones have also been established in fungi by Burgeff in *Mucor mucedo*, by Raper in Achlya and by Zickler in Bombardia.

In contrast with the review of Lewin I believe to have furnished proof that the phenomena of general *bipolar bisexuality*, of *bisexual potency*, of *relative sexuality* and the *presence of gamones* are not to be looked upon as a "theoretical anthropomorphic concept" (Lewin), but that they are well founded on facts which are of decisive significance for a general theory of fertilization and sexuality.

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ZONAL DISTRIBUTION OF VERTEBRATES IN A MEXICAN CLOUD FOREST[1]

PAUL S. MARTIN

Museum of Zoology, University of Michigan

INTRODUCTION

As a means of generalizing the abundant information on tropical animal distribution, and as an approach to the problem of faunal origin, the hypothesis of faunal zonation holds great promise. If certain animals prove to be restricted to a given major tropical environmental type, such as tropical rain forest or tropical thorn forest, we can map faunal dispersal routes in terms of the modern distribution of these habitats, consider questions concerning historic contact between habitat islands presently isolated, and appraise more effectively the origin of their faunas. Studies on isolated cloud forest in the Sierra de Tuxtla (Wetmore 1943), dry pine-oak forest in The Sierra de Tamaulipas (Martin, Robins, and Heed 1954), and arid tropical valleys surrounded by humid forests in Atlantic drainage of Chiapas and Guatemala (Stuart 1954) illustrate local biogeographic problems of this type. The monumental studies of Chapman (1917, 1926, 1931) on the relationship between distribution of the major tropical forest types and their associated avifaunas in northern South America illustrate on a much larger scale the potential of this method. Such analyses depend largely on a thorough knowledge of the local distribution of the vegetation zone in question and some information on the fidelity and constancy which certain animals exhibit to it. Although complete habitat restriction of a polytypic species may not be expected throughout its range, apparent habitat preferences can often be recognized, especially on a local basis.

Rather than examine the assumptions, limitations and possible results of zonal studies, I will discuss just one of the major tropical habitats used in zonal analysis, the cloud forest, and consider the pattern of vertebrate distribution along an altitudinal gradient through a single segment of this habitat. Results of a transect study begun in 1948 show a low level of faunal restriction to cloud forest at its latitudinal limit, a pattern not reported elsewhere. Some of the problems encountered in the analysis of this transect will be illustrated.

MEXICAN CLOUD FOREST

Cloud forest is a part of the world plant formation termed Temperate Rainforest (Schimper 1903); elsewhere the names Montane Rainforest, Fog Forest, Upland Rainforest, Elfin Woodland, Low Evergreen Forest (Selva Baja Siempre Verde of Miranda 1952), Subtropical Zone and Humid Upper

[1]Presented at a symposium on the Origin of the Biota of Middle America, sponsored by the American Society of Naturalists, Gainesville, Florida, Sept. 1954.

Tropical Zone, are all used variously to apply to mild or cool, generally forest free, humid areas of uniform temperature in mountainous parts of the New World tropics. Except in certain insular situations such as the Greater Antilles, cloud forest is generally considered to harbor a rich and distinctive fauna. Among the birds the following number of species are listed: 230 in Colombia (Chapman 1917), 222 in Ecuador (Chapman 1926), 39 in El Salvador (Dickey and Van Rossem 1938), 39 in Guatemala (Griscom 1932) and 55 in Mexico (Goldman 1951). Of the mammals 34 are listed for Panama (Goldman 1920) and 46 for Mexico (Goldman 1951). While discrepancies have been recognized (see especially Griscom 1932, p. 56), most of the species listed by the above authors are thought to be reliable indicators of Cloud Forest conditions.

Although no thorough vegetational survey of Mexican cloud forest is available to relate to the pattern of animal distribution, Miranda and Sharp (1950) describe many important areas, and an excellent account of Chiapas has appeared (Miranda 1952). In his brief review of Mexican vegetation types, Leopold (1950) notes that cloud forest is relatively small, covering less than one per cent of the land surface of the republic. Its climate is mild or cool, with slight temperature range both daily and seasonally; frosts are infrequent or unknown; rainfall is moderate to heavy; the relative humidity is generally high, even during the dry season, and at all seasons ground level cloud banks or fogs may be expected. Under these climatic conditions a mesic, broad-leaved, evergreen or semideciduous forest of varying height will develop best characterized by its wealth of epiphytes. Despite the small area occupied by cloud forest, its flora is rich and diverse; some of the more typical elements include Podocarpus, tree ferns, and sweet gum (Liquidambar). In Mexico many genera with temperate as well as tropical centers of distribution occupy these areas (Miranda and Sharp 1950) in contrast to the vertebrate fauna, which is predominately tropical. On a regional basis cloud forest acts as a faunal highway for many animals, but in areas where its distribution is fragmented, such as Mexico (figure 1), faunal movement between isolated tracts of forest is clearly reduced. Elsewhere brief descriptions of various Middle American cloud forests may be found in the following sources: Beard 1944, Carr 1949, 1950, Lauer, 1954, Holdridge 1953, Standley 1945 and Standley and Steyermark 1945.

TAMAULIPAN CLOUD FOREST

With this brief background we may turn to a more detailed survey of Middle American cloud forest at its latitudinal limit, 23° north, in the southwestern part of the State of Tamaulipas, about 7 kilometers northwest of Gómez Farías (figure 1). Here a tract best known as Rancho del Cielo covers roughly 150 square kilometers on the eastern slopes of the Sierra Madre Oriental. Arid tropical lowlands and foothills covered with arid or subhumid forests isolate this region from the nearest similar area of cloud forest at Xilitla in San Luis Potosí about 150 kilometers to the south. Between Xilitla

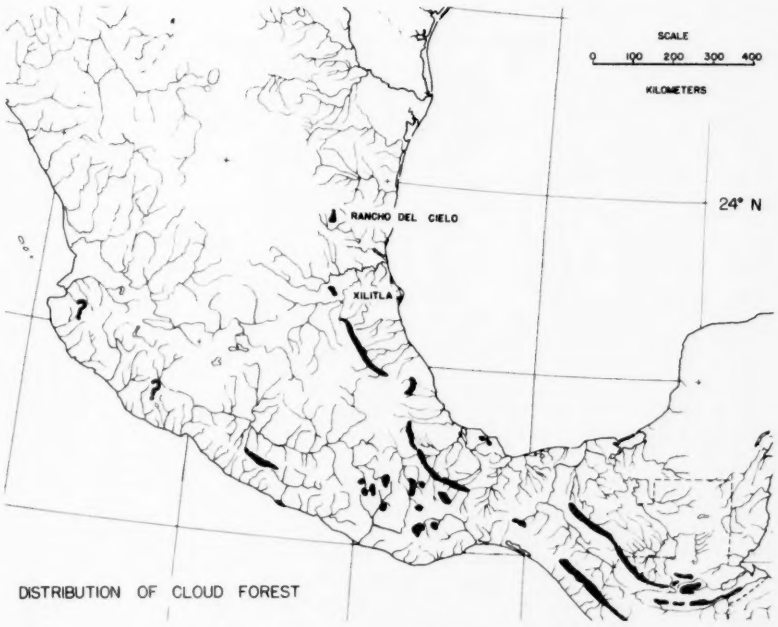

FIGURE 1. Distribution of cloud forest in the northern part of Middle America, modified from Leopold (1950). Possible outposts on the Pacific slope of Mexico indicated by question marks.

and Rancho del Cielo no humid montane forests of either pine-oak or true cloud forest have been reported, although at an elevation of 1140 meters east of Ciudad del Maiz Davis and Johnston (1947) mention a subhumid forest of Quercus, Liquidambar and Cercis. In the following three features the Rancho del Cielo forest may be characterized rather precisely.

1. Climate. No standard meterological data are available from Tamaulipan cloud forest; nonetheless the following qualitative observations based primarily on records kept by William Francis Harrison of Rancho del Cielo describe some of the climate features. The annual mean temperature is close to 19°C. with a mean seasonal variation of about 5°C. Daily ranges in winter are greater than in summer. Although light frosts may be expected annually, only one severe freeze, which lasted three days in February 1951 with a minimum of minus 6°C on February 1, is reported by Mr. Harrison in 19 years of residence in this region.

The annual rainfall probably averages more than 2000 mm. with an estimated 1700 mm. falling in the unusually dry year of 1953. Although precipitation fluctuates seasonally, the dessicating influence of the spring dry season is much modified by high humidity and frequent fogs. In the dry as well as the wet season these are an outstanding climatic feature of the

PLATE 1. Primaeval cloud forest near Rancho del Cielo, July 1950, photographed by Charles F. Walker.

area. Reduced evaporation, rather than any appreciably greater rainfall, probably accounts for the rich mesophytic aspect of the cloud forest in contrast to adjacent forest types.

2. Vegetational Structure. Except for bare or shrub-covered rock outcrops the forest is uniformly tall, dense, and compact, its canopy between 20 and 30 meters with occasional trees reaching 40 meters. The trunks are closely spaced, straight, clean boled, narrow crowned, and festooned, if not overburdened with a variety of epiphytes (plate 1). A quantitative index of density was obtained by Shanks (1954) who recorded a mean basal area of 102 square feet per acre by plotless sampling of four of the dominant trees more than 6 inches in diameter. During the winter dry season some of the canopy trees, notably Acer, Liquidambar and Cercis, lose most of their leaves, but the majority do not, and the forest maintains a semi-evergreen aspect.

3. Flora. The upper and lower distributional limits of certain dominant trees including the genera Podocarpus, Liquidambar, Clethra, and *Quercus sartorii* effectively delimit the cloud forest boundary. These trees occur throughout the cloud forest but not above or below it. In addition many other plants including Acer, Fagus, Illicium, Meliosma, Eugenia, Hamamelis, Rhamnus, Persea, and Piper also serve as plant indicators although their local distribution is more restricted and does not always reach the boundaries of the habitat. Of the total flora reported by Miranda and Sharp (1950) from cloud forest in eastern Mexico, a large part is found at Rancho del Cielo, some notable exceptions being Nyssa, Weinmannia, and tree ferns of large size. For further description of the Rancho del Cielo forest see Sharp et al 1950, Hernandez et al 1951, Sharp 1954, and especially Harrell 1951.

Cloud forest at Rancho del Cielo reaches its altitudinal limits at 900 and 1700 meters elevation with typical areas mainly between 1000 and 1500 meters. Beyond these levels it is replaced below by subhumid tropical deciduous forest and above by humid pine-oak forest (figure 2). The former differs drastically in flora sharing few tree species, mainly an oak, *Quercus germana*, and a palm, *Chamaedorea*, with the cloud forest; its structural aspect is totally different. The humid pine-oak forest shares more woody genera, including Magnolia, Carpinus, Abies, Ternstroemia, and Carya, as well as more of the structural features. The pine-oak area differs chiefly in lower density of trees, a partly open canopy, and a higher frequency of sclerophylls. The diagram illustrated in figure 2 represents vegetation types as observed before 1952 when the mountainside from 300 meters to the top of the peaks west of Rancho del Cielo (2400 meters) was largely mantled with primeval forest. The present faunal survey is based on altitudinal records obtained mainly before recent lumbering and other destructive activities seriously affected the area.

I have stressed environmental features to emphasize the unique aspect of Rancho del Cielo locally. It is the only known tract of cloud forest in the State of Tamaulipas. Also I have sought to establish features of similarity to other cloud forest in Mexico. Under such circumstances would we not

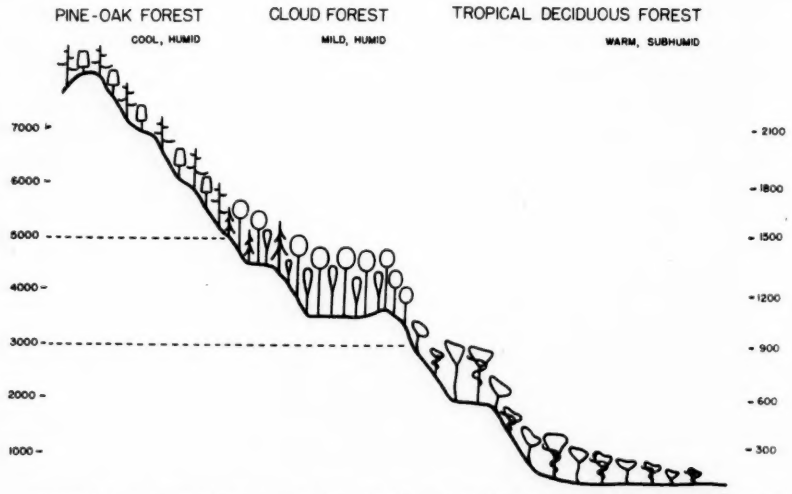

FIGURE 2. Vegetation profile through the Sierra Madre Oriental at lat. 23° north in southwestern Tamaulipas; highly diagramatic. Elevation given in feet on the left, meters on the right. Altitudinal limits of cloud forest indicated by broken lines.

expect a distinctive indigenous subtropical zone fauna? The search for such a faunal element prompted the first studies at Rancho del Cielo, but after several seasons of field work in which many of the animals first thought to represent an indigenous element were found commonly outside the cloud forest, this view was abandoned.

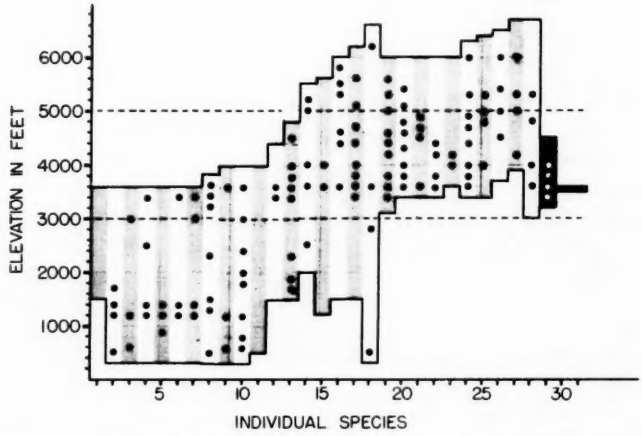

FIGURE 3. Altitudinal distribution of 31 reptiles and amphibians found in the the Rancho del Cielo cloud forest, as listed in table 1. The upper and lower limit of each species is represented by a vertical bar, additional altitudinal records indicated by dots. Species found only within the cloud forest, between 3000 and 5000 feet, are shown in black.

ALTITUDINAL LIMITS OF THE VERTEBRATE FAUNA

A total of 97 resident species of terrestrial vertebrates have been collected or recorded at Rancho del Cielo to date (table 1) representing field

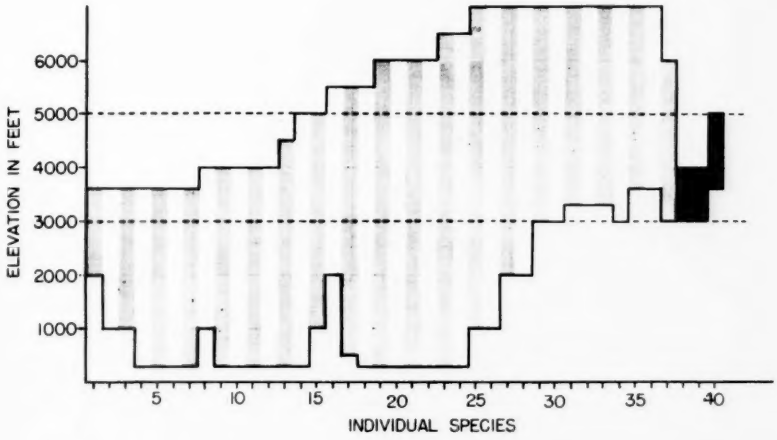

FIGURE 4. Altitudinal distribution of 40 breeding birds of the Rancho del Cielo cloud forest, as listed in table 1. The upper and lower limit of each species is represented by a vertical bar; those found only inside the cloud forest are shown in black.

work over a six year period at all seasons by numerous collectors. The altitudinal limits of this fauna based on records along the gradient illustrated in figure 2 are summarized in figures 3–5. Although the highest peaks of the Sierra Madre Oriental reach 2400 meters in this area, no collections have been made above 2100 meters (7000 feet). Upper and lower limits of the cloud forest are shown by the dashed lines at 900 and 1500 meters (3000 and 5000 feet) in figures 2–5; within this range any species characteristic of a Subtropical Zone should appear. Such animals are shown by solid black bars and are listed under section D in table 1. Trinomial names are not used as they would contribute little information to the problem at hand. In this region of porous karst limestone there is no permanent surface drainage beyond a few small isolated springs and thus the vertebrate fauna is exclusively terrestrial. A discussion of the distinctive cloud forest forms follows.

1. Birds (figure 4). In addition to unpublished field notes altitudinal records were obtained from Harrell (1954), Sutton and Pettingill (1942), Eaton

TABLE 1

ALTITUDINAL DISTRIBUTION OF CLOUD FOREST VERTEBRATES, IN NUMERICAL ORDER AS LISTED IN FIGURES 3–5

I. Reptiles and amphibians, (figure 3)	II. Birds, (figure 4)	III. Mammals, (figure 5)
A. Lowland component, dispersal centers mainly in tropical deciduous forest		
1. *Tantilla rubra*	1. *Piranga leucoptera*	1. *Sigmodon hispidus*
2. *Leptodeira annulata*	2. *Veniliornis fumigatus*	2. *Artibeus cinereus*
3. *Thalerophis mexicanus*	3. *Campylopterus curvipennis*	3. *Desmodus rotundus*
4. *Bothrops atrox*	4. *Chaetura vauxi*	4. *Oryzomys fulvescens*
5. *Drymobius margaritiferus*	5. *Phloeoceastes guatemalensis*	5. *Pecari tajacu*
6. *Bufo valliceps*	6. *Turdus grayi*	6. *Didelphis marsupialis*
7. *Bufo horribilis*	7. *Arremonops rufivirgatus*	7. *Dasypus novemcinctus*
8. *Syrrhophus cystignathoides*	8. *Sittasomus griseicapillus*	8. *Neotoma angustapalata*
9. *Hyla baudinii*	9. *Piaya cayana*	9. *Centurio senex*
10. *Sceloporus variabilis*	10. *Otus guatemalae*	10. *Nasua narica*
11. *Hyla miotympanum*[1]	11. *Momotus momota*	11. *Sciurus deppei*
12. *Eleutherodactylus hidalgoensis*[1]	12. *Thryothorus rutilus*	
13. *Syrrhophus latodactylus*[1]	13. *Icterus graduacauda*	
	14. *Xiphorhynchus flavigaster*	
	15. *Turdus assimilis*	
B. Wide ranging component, found both above and below cloud forest		
14. *Crotalus durissus*	16. *Cyclarhis gujanensis*	12. *Felis onca*
15. *Tropidodipsas sartorii*	17. *Piranga bidentata*	
16. *Eumeces dicei*	18. *Xanthoura yncas*	
17. *Chiropterotriton multidentata*	19. *Cryptur ellus cinnamomeus*	
18. *Rana pipiens*	20. *Leptotila verreauxi*	
	21. *Ciccaba virgata*	
	22. *Piculus aeruginosus*	
	23. *Penelope purpurascens*	
	24. *Catherpes mexicanus*	
	25. *Myadestes obscurus*	
	26. *Dactylortyx thoracicus*	
	27. *Catharus mexicanus*	
	28. *Myiarchus tuberculifer*	

TABLE 1 (*continued*)

I. Reptiles and amphibians, (figure 3)	II. Birds, (figure 4)	III. Mammals, (figure 5)
C. Montane component, dispersal centers mainly in oak-pine forest		
19. *Rhadinaea crassa*	29. *Melanotis caerulescens*	13. *Peromyscus ochraventer*
20. *Pseudoeurycea scandens*	30. *Lepidocolaptes affinis*	14. *Mazama sartorii*[2]
21. *Pseudoeurycea bellii*	31. *Trogon mexicanus*	15. *Peromyscus boylei*
22. *Pseudoeurycea cephalica*	32. *Pachyramphus major*	16. *Ursus americanus*
23. *Geophis semiannulatus*	33. *Vermivora superciliosa*	
24. *Chiropterotrition chondrostega*	34. *Hesperiphona abeillei*	
25. *Abronia taeniata*	35. *Melanerpes formicivorus*	
26. *Thamnophis mendax*	36. *Atlapetes pileatus*	
27. *Crotalus lepidus*	37. *Amazilia cyanocephala*	
28. *Lepidophyma flavimaculata*[2]		
D. Apparent indigenous cloud forest component		
29. *Pliocercus elapoides*	38. *Glaucidium minutissimum*	17. *Cryptotis mexicana*
30. *Amastridium sapperi*	39. *Lampornis amethystinus*	18. *Glaucomys volans*
31. *Sceloporus cyanogenys*	40. *Vireo gilvus*	19. *Marmosa mexicana*
		20. *Enchisthenes hartí*
		21. *Lasiurus cinereus*
		22. *Sylvilagus gabbi*
		23. *Oryzomys alfaroi*
		24. *Reithrodontomys megalotis*
		25. *Reithrodontomys mexicanus*
		26. *Felis glaucula*

[1]On the basis of their distribution elsewhere in Mexico these species may have reached Tamaulipan cloud forest via a montane forest route.
[2]On the basis of their distribution elsewhere in Mexico these species probably reached Tamaulipan cloud forest via a lowland tropical forest route.

and Edwards (1948) and Robins and Heed (1951). Of a total fauna of 40 breeding birds, only three have not appeared outside the cloud forest, and of these, two, *Glaucidium minutissimum* and *Lampornis amethystinus*, definitely are not restricted to cloud forest elsewhere. The former reaches sea level in the Rio Balsas Basin of Guerrero while the latter occurs in temperate forests as high as 10,000 feet in several portions of its Mexican range. *Vireo gilvus* (*amauronotus* group) may represent a true cloud forest indigene, although its apparent restriction to Tamaulipan cloud forest is based on only three specimens and less than 10 sight records. Two species generally considered cloud forest forms elsewhere in Mexico and Guatemala, *Catharus mexicanus* and *Dactylortyx thoracicus*, are more numerous in Tamaulipan cloud forest than in adjacent forest habitants, but both were noted repeatedly in the breeding season in tropical decidous forest of the lower mountain slopes and in the oak-pine forest to at least 7000 feet. *Catharus mexicanus* has been collected in the breeding season in dry oak-pine forest in the Sierra de Tamaulipas, an area completely isolated from Tamaulipan cloud forest (Martin, Robins and Heed 1954).

2. Reptiles and Amphibians (figure 3). A collection of over 2500 specimens of reptiles and amphibians from southwestern Tamaulipas is the basis for distributional records. Those of special interest are listed and disdiscussed in part in a separate paper (Martin 1955). In addition to extreme altitudinal records as shown by the bars, intermediate records are plotted (black dots) thus giving a better index of local distribution than can be shown by the extreme records alone. Of a herpetological fauna of 31 species, only three are unrecorded outside the cloud forest. Two of these,

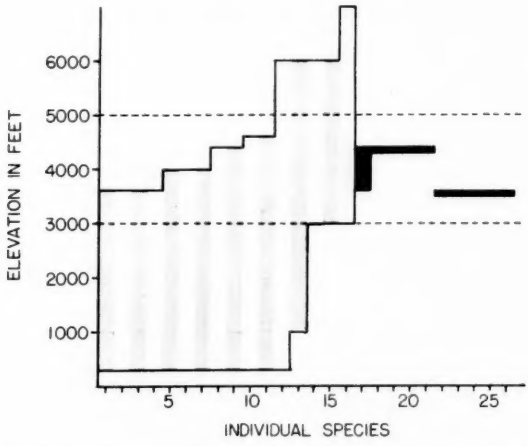

FIGURE 5. Altitudinal distribution of 26 species of mammals found in the Rancho del Cielo cloud forest, as listed in table 1. The upper and lower limit of each species is represented by a vertical bar; those found only inside the cloud forest are shown in black.

Amastridium sapperi and *Sceloporus cyanogenys*, were found only at 3600 feet. *Amastridium* is too rare for zonal analysis while *S. cyanogenys* is found commonly on the arid coastal plain and foothills of northeastern Mexico and south Texas and is unreported from cloud forest areas elsewhere. The Rancho del Cielo population represents an endemic subspecies. The third apparently indigenous species, a snake of the genus Pliocercus, is represented by 48 specimens from various altitudes in the cloud forest; however, elsewhere in eastern Mexico it is equally typical of lowland rain forest. Thus none of these three species definitely can be considered distinctive cloud forest forms throughout their ranges. In addition the local distribution of only one, Pliocercus, is known well enough to make its discovery outside the cloud forest appear relatively improbable.

3. Mammals (figure 5). This group is the least known. Localities listed by Hooper (1953), Baker (1951), de la Torre (1954), and Goodwin (1954) are the main sources for altitudinal records. Of a mammalian fauna of 26 species nine were collected only in the cloud forest, and of these, four are considered typical of cloud forest (humid upper tropical zone) elsewhere in Mexico (Goldman 1951). A strong collecting bias in favor of the cloud forest fauna undoubtedly accounts for this high number of apparent indigenes. Many of these may be expected in the humid oak-pine forest which is virtually unsampled. I see no reason for anticipating a higher indigenous element among the mammals than is currently recognized for the better known birds and reptiles. To date none of the nine except *Cryptotis mexicana* are known from more than a single locality in this region.

CLOUD FOREST AS A FAUNAL BARRIER

If we conclude that a very small number of animals are actually confined to Tamaulipan cloud forest, including some supposedly typical of this zone further south, can we make any positive statement about the habitat as a faunal boundary? Are many animals within reach of its borders excluded from the cloud forest? Among the birds there are at least 15 common, readily identified species in the pine-oak area which do not breed in the cloud forest. These include *Basileuterus belli*, *Tanagra musica*, *Piranga flava*, *Columba fasciata*, *Dendrocopos villosus*, *Troglodytes brunneicollis*, *Ptilogonys cinereus*, *Mitrephanes phaeocercus*, *Catharus occidentalis*, and *Aphelocoma ultramarina*. The latter is so typical of the pine-oak woods that we came to call the upper edge of the cloud forest the Aphelocoma line. At the lower edge the common birds which are heard up the mountainside through the tropical deciduous forest until the cloud forest level is reached number more than 15, including *Parula pitiayumi*, *Habia rubica*, *Euthlypis lachrymosa*, *Columba flavirostris*, *Basileuterus culicivorus*, *Parus atricristatus*, *Amazilia yucatanensis*, *Cynanthus latirostris*, and *Polioptila caerulea*. Some of these are reported as stragglers in the cloud forest (Harrell 1951), but their frequency drops off sharply at the edge of the tropical deciduous forest. Most of the reptiles and amphibians are less obvious and are therefore less useful as indicators; nonetheless, two, *Sceloporus tor-*

quatus and *Sceloporus grammicus*, are so abundant in the oak-pine area that their absence in the cloud forest is conspicuous. Finally, a few species such as *Trogon ambiguus* and a lizard, *Gerrhonotus liocephalus*, occur in subhumid forest habitats both above and below, but completely avoid the cloud forest. Thus it appears that the boundaries of this environmental type are influential in determining animal distribution.

REGIONAL ORIGIN OF THE CLOUD FOREST FAUNA

Despite its rich indigenous flora and distinctive structural development the preceding account indicates that the Rancho del Cielo cloud forest lacks a comparable vertebrate fauna. Rather than a restricted component with its closest relatives in other cloud forest environments farther south in Mexico, as is the case of the dominant floral elements, the Rancho del Cielo fauna appears to have been derived from the two main environmental types presently in contact with the cloud forest, a subhumid tropical lowland fauna below (section A, table 1) and a humid temperate fauna from above (section C, table 1). Many animals inhabiting each of these areas do not enter the cloud forest, which is composed of only a partial mixture of their faunas. Those species found both above and below cloud forest (section B, table 1) have centers of abundance on at least one side, or in the case of some wide ranging forms as *Rana pipiens* and *Catherpes mexicanus*, on both sides. A small group including *Catharus mexicanus*, *Dactylortyx thoracicus* and perhaps some of the species of section D, table 1, may represent a true cloud forest element in Tamaulipas.

Those animals reaching Tamaulipan cloud forest via the tropical deciduous forest follow a broad belt of this vegetation type northward through the lowlands from central Veracruz to extreme eastern San Luis Potosí and southern Tamaulipas (see Leopold 1950). Those reaching Tamaulipas via the humid and subhumid pine-oak zone follow a narrow forest band extending southward with several important gaps in San Luis Potosí to the elevated southern portion of the Mexican Plateau, and continuing northward from western Tamaulipas along the higher, wetter parts of the Sierra Madre Oriental to the vicinity of Saltillo, Coahuila.

DISCUSSION

To end the review of Rancho del Cielo we may consider some of the aims and limitations of this type of transect study. To my knowledge no similar survey has been made through other undisturbed cloud forest areas, and the chief difficulty in interpreting the results obtained in Tamaulipas lies in this lack of comparable data. Admittedly a project of this type requires a long field work period and the cooperation of many collectors. Compared with the more conventional biotic survey methods in which many specimens are assembled from a wide variety of environments in a large geographical area, this approach is much less productive of distributional records and undescribed animal populations. However, if a realistic picture is to be drawn of cloud forest as a unique animal habitat, similar transect studies

are needed to demonstrate what species, if any, are true cloud forest endemics.

Employing the transect method in the Rancho del Cielo region we find that despite an apparently suitable cloud forest habitat, the pattern of vertebrate distribution does not reveal a strong indigenous subtropical zone fauna, comparable to the flora. For several reasons an atypical pattern might be expected at this latitude. Northern Mexico is far from the source of supply of subtropical zone animals, best developed in Colombia and Ecuador, with a secondary center of evolution in nuclear Middle America. In addition cloud forest is not especially extensive this far north and can not support very large breeding populations of the less common animals. Although the Rancho del Cielo fauna is comprised largely of tropical animals at or near their known northern limit, most of these appear to have reached southern Tamaulipas via other avenues of habitat than the cloud forest. The Tamaulipan cloud forest fauna has its origin in animals living today in adjacent zones rather than animals inhabitating the same zone southward at a comparable altitude. On a faunal basis there is little distinctive about Tamaulipan cloud forest; only when flora and vegetation are taken into account does the area show unmistakable cloud forest characteristics.

Apparently the area lacks many typical cloud forest faunal elements as recognized further south; certainly this is true of the group with the greatest potential for bridging habitat gaps, the birds. Of 39 species listed by Griscom (1932) in Guatemala, 34 do not reach Tamaulipan cloud forest, and of 55 Mexican species listed by Goldman (1951), 44 were not found. Assuming that these forms exhibit a higher level of fidelity to cloud forest than those found at Rancho del Cielo, and assuming the criteria used above to define the cloud forest environment in Tamaulipas are suitable, the following hypothesis is advanced to account for the observed faunal situation.

Tamaulipan cloud forest has long been out of contact with similar environments further south, such as that at Xilitla, San Luis Potosí. Climatic changes in this interval, possibly during the xerothermic or thermal maximum period, if not during interglacial times, reduced the present size of the cloud forest considerably. The floristic elements were driven into humid pockets and ravines in the mountains, while the fauna was gradually reduced to those species able to maintain sizeable breeding populations by occupying adjacent, less mesic, forest habitats. In this manner a distinctive indigenous cloud forest faunal element was lost while most of the distinctive floristic elements were retained.

The above discussion will gain substance only if further transect studies show that the many allegedly typical cloud forest animals listed by Griscom, Goldman and others actually are of high indicator value, and that they do not use adjacent vegetation types as a faunal pathway. In the absence of similar transect studies in areas of primaeval forest southward, I assume the Rancho del Cielo pattern is unique, but such studies are needed for a more realistic treatment of the origin of the Tamaulipan cloud forest fauna as well as a thorough evaluation of the zonation concept.

SUMMARY

A very useful method of analysis in the study of faunal origin is based on the relationship of patterns of animal distribution to that of major vegetation types. Application of this method in a zonal analysis of cloud forest at its its northern latitudinal limit in eastern Mexico yielded the following observations:

1. Presence of a small but distinctive forest habitat, humid at all seasons, with a mild mean annual temperature of slight seasonal variation.

2. A unique indigenous floral assemblage including genera with both temperate and tropical centers of distribution, many of these found in Middle America only in the cloud forest.

3. A vertebrate fauna derived almost entirely from that of adjacent forest habitats, lacking most of the indigenous forms typical of cloud forest further south in Mexico and Middle America.

The contrast between a rich indigenous cloud forest flora, actually richer than many similar areas further south, and a locally derived fauna lacking such distinctive elements suggests that the area has not been in recent contact with similar forests to the south. Possibly climatic change in this interval depleted the vertebrate fauna far more than the associated flora and left the habitat open to occupation by those animals occupying adjacent vegetations zones.

ACKNOWLEDGEMENTS

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CELLULAR BIOLOGY AND "TISSUE CULTURE"

PHILIP R. WHITE

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Forty years ago it was freely predicted that the then newly introduced method of "Tissue Culture" would surely revolutionize our understanding of body structure and function. It has not done so, and more recently there has been a tendency to swing to the other extreme, to emphasize this failure and to forget the very real contributions which the method has made. Since there is some danger that young blood, which is so essential to the continued development of any field of endeavor, may be turned away for lack of realization of the potentialities of this one in particular, it has seemed worth while to try to clarify the picture somewhat.

The term "Tissue Culture" (I prefer "Cell Culture" which I believe to be more accurately descriptive) designates a rather wide range of methods all of which have a single objective—to isolate a cell or group of cells from a complex organism, either animal or plant, to place these cells in an artificial environment designed to approximate the essential features of the normal conditions within the body, and thus to maintain and study them in a living and actively metabolizing state. This will commonly include their reactions to controlled changes in the environment. On the positive side such controlled changes involve the addition of influences to which cells in the body are not normally subjected. On the negative side, they involve deletions or modifications of influences normally coming from surrounding cells or cell systems. One can thus sort out the effective from the merely fortuitous elements of cellular ecology and define them. One can, as Haberlandt stated in 1902 "Cast many interesting sidelights on the peculiarities and capacities which the cell, as an elementary organism, possesses (and can draw) conclusions concerning the relationships and reciprocal influences to which the cell is subjected within the multicellular organism" (translation by P. R. W.). Any procedure which has these objectives is a part of what we call "Tissue Culture." The use of tissue cultures differs from the use of tissue slices or homogenates in insisting on maintenance of *capacity for continued growth* and in placing emphasis in general on cells as individuals, at least in theory, rather than on a statistical resultant of the combined activities of a mixed collection of cells.

The methods of tissue culture are many, with many specific purposes. They are by no means restricted to the hanging drop cultures and specialized flask cultures which are their most widely familiar representatives. There are cultures of organs, for example, bones, eyes, teeth, parathyroids, and of plant embryos and root-tips, carried out in watch glasses of several different types. There are cultures of disaggregated whole embryos, or of

tissues disorganized by partial digestion with trypsin, grown in sponge matrices or on the inner surface of roller tubes, or on metal grids or paper tables. There are cultures of lung and of nasal mucosa made in perfusion chambers for pharmacological studies where ciliary activity can be followed under experimental treatment. There are cultures made in Erlenmeyer flasks, of animal cells for the propagation of viruses and of plant roots for studies of special metabolic problems. There are cultures of skin on "flying cover glasses" in roller tubes or sitting tubes, for studies on keratinization and for examination of nuclear behavior. There are cultures of plant tumor tissues on agar slants for studies of the etiology of cellular malignancy. And there are the classic types of Carrel-flask and hanging-drop cultures for a variety of purposes, ranging from the diagnosis of obscure tumors and study of brain organization and the pulsatile activity of neuroglia to collagen formation, the spontaneous development of malignancy in animal cells and details of cellular nutrition. There is hardly a branch of science dealing with the cellular biology of higher animals and plants which has not profited in the past few decades from some sort of "tissue culture" study.

I suspect that here, indeed, lies one reason why the field is not more generally appreciated: it includes so many different sorts of studies that their common element, the tissue culture approach, may often pass unrecognized. The recently published "Bibliography of Research in Tissue Culture" prepared for the Tissue Culture Association, covers no less than 15,000 references appearing in 1035 different journals, representing scores of different technical specialties: microscopic and comparative anatomy, general and cellular physiology, biochemistry both plant and animal, pharmacology, endocrinology, pathology, virology, oncology, nutrition, clinical diagnosis, radiology, biophysics and a dozen more. For each of these a different specialized technique has had to be developed, but in none is the technique itself of primary interest; all are bound together by the common objective of isolating a cell, tissue or organ, keeping it alive and in a healthy state and using it to solve some particular problem of cell biology.

Each of us has his own range of interests. If I were asked to name some outstanding contributions of tissue culture to biological science (and I am quite sure that no two workers would give the same list) I would undoubtedly start with Harrison's demonstration that the neurofibrils seen in histological section in all animal tissues do not arise within the tissues traversed, nor at the endorgans, but by outgrowth from the nerve cell itself. Obvious as this seems today it was a bitterly controversial question fifty years ago and was only settled by growing the nerve cell *in vitro* (1907). Second, I would cite the general conclusion that animal tissues are, through the potential continuity of cell generations, capable of immortality, death being a failure of integration rather than of cellular senescence. This was Carrel's great contribution over and above a multitude of technical details (1912–1939).

But having made these two concessions to the more remote past I would skip over a good many years. And I must here digress a bit to provide a word of explanation, lest what I have to say may be too greatly misunderstood. I am writing this paper largely for an American audience and am consequently emphasizing the picture as one saw it from this side of the Atlantic. In the United States the field was largely dominated up to 1945 by the name of Carrel. When Americans thought of tissue culture they thought of Carrel, Parker, Baker, Ebeling, Fischer. The only other American "school" was that of Lewis. In Europe there was a number of other groups which had no direct connection with Carrel, notably three in England centering around Strangeways, Fell and Canti; Willmer, Trowell and Jacoby; and Drew, and extended, on the continent, in the persons of Gaillard and Martinovitch; an Italian group centering around Levi and Olivo, who were rather closely linked to Carrel; and Maximov in Russia who later came into the Lewis orbit. From about 1925 onward the Strangeways group has continually added significantly to our store of knowledge. But their work has been characterized, in the American mind, by the emphasis on *organs* rather than tissues and cells, in spite of the cell studies of Canti, Hughes, Willmer, Jacoby and others, and as such has for many years had little impact on American "tissue-culture" thought. That has been our loss, as we have come to realize more recently. But it has been a part of the picture. Be that as it may there was undoubtedly in America a period when those who dominated the field of "Tissue Culture" were prone to constitute themselves a closed corporation by emphasizing the difficulties inherent in the art; and in this isolation they perhaps permitted themselves to be somewhat more fascinated by the techniques than by their proper objectives.

I would go first to 1931, to Lewis' demonstration that water and insoluble solid matter are taken into the cell, not by physico-chemical absorption at the cell surface, nor by phagocytosis in the usual sense, but by the cell casting out a membranous "net" and sweeping these metabolites into its body *en masse*, by what he called "pinocytosis." Lewis' name and that of his wife had, indeed, been closely bound up with the idea of tissue culture since its beginning (1911) but their earlier contributions on cell nutrition were in many respects "ahead of their time," applying a point of view for which adequate methods and basic knowledge were not yet available. This earlier work has left little concrete to which one can point:—their point of veiw on cell nutrition is only today becoming technically effective.

Then, if my readers will forgive a personal note, I would pass to 1934, to the demonstration that the root of a plant is capable of autonomous existence, being dependent on the plant as a whole for certain metabolites, first and most obviously for carbohydrate but second and far less evidently for what subsequently proved to be thiamin and other vitamins (Bonner, 1939; Robbins, 1939; White, 1939). These can be supplied in an artificial environment. From this beginning has come a great deal of our present understanding of root-cell function including the function of water excretion.

I would follow with Gaillard's demonstration (1940) that the juvenile human parathyroid (and the ovary and other endocrines) can be so modified by cultivation in a nutrient containing serum of a prospective recipient that it loses its original histochemical specificity and can be successfully transplanted. Although not yet perfected to the point of being generally clinically practicable this has obviously very great possibilities.

Shortly thereafter come two contributions from a totally different field, Dawson's demonstration (1941) that nicotine is not synthesized in the tobacco leaf where it reaches its highest concentration, but in the root, where one finds it in only trace amounts; and my own demonstration, with Braun (1941), that the cells of crown-gall tumors of sunflowers, periwinkles and other plants acquire a real and permanent malignant quality which is not, as formerly thought, dependent on the continued presence of an external agent (bacteria) but involves an alteration in intrinsic metabolic pattern characterized by a clear decrease in specialization (Gautheret, 1950). At about the same time (1941) and again in still a different field, Murray and Stout demonstrated that certain clinically obscure neuromas which are masked by the variety of non-tumor cells of the stroma, can be diagnosed by growing the tumor cells in tissue culture and eliminating the stromal elements.

I would cite the observation that D.D.T. (W. H. Lewis and A. G. Richards, 1945) and certain clinically carcinolytic agents (J. R. McConnell, S. F. Hallett and M. J. Shear, 1947) are without evident cytotoxic effect on cells grown *in vitro*, whereas the antihistaminics exert their clinical effects by direct means, dependent on real cytodynamic properties (Pomerat et al, 1953). I would cite the demonstration that, in particular artificial environments, hence possibly also in the body, certain amino-acids may mediate growth without differentiation while others may bring about differentiation without increase in mass (Wolff et al, 1950). I consider of great import the fact demonstrated by Willmer (1941) and extended by Moscona (1952) that a tissue can be broken down to its constituent cells by trypsin digestion and that when these cells are again brought together certain tissues such as cartilage will reconstitute themselves showing that the histological potency is established quite early in development.

I would also cite Fell and Mellanby's demonstration (1953) that keratinization of epithelium is prevented by high levels of Vitamin A nutrition so that the character of the dominant cellular pattern, whether ciliated or keratinized, can be changed by altering the nutrient constituents of the bathing fluid. I like also Pomerat's demonstration (1951) that neuroglial cells maintain an active, rhythmic, pulsatile movement which may prove to be a factor in maintaining cellular metabolism in poorly vascularized tissues, and that of Hsu (1954) that some cases of pseudohermaphroditism in which the true sex is clinically uncertain, can be quickly and accurately diagnosed by first growing non-specialized cells (skin) *in vitro* and then swelling the nuclei by treatment with hypotonic solutions by the method of

Hughes (1952). Nor should one pass over the use of cultivated cells both as substrata in which to propagate a variety of viruses (Levaditi, 1913; Maitland, 1928; Enders et al, 1952) and for the differential diagnosis of related virus strains (J. T. Syverton and W. F. Scherer, 1954). I have also left uncited the slow and laborious studies of cell nutrition in both plants and animals which are being carried on constantly, and which, I am certain, are contributing and will continue to contribute to a clearer understanding of how cells function as the basic physiological units in all complex organisms.

These are not the results of applying *a* technique but rather a multiplicity of techniques. Vitamin A function and gland adjustment are studied in watchglass cultures, antihistaminic effects in perfusion chambers, nicotine synthesis in Erlenmeyer flasks, pinocytosis in hanging drops, plant tumor-cell specialization on agar slants or filter paper tables, virus cultures in prescription bottles, sitting tubes or roller tubes, cartilage differentiation in watch-glasses, skin specialization on cover-slips in roller tubes. Each method has been devised for a particular group of problems; they have only one thing in common—that they are all dealing with questions of cellular ecology and tissue metabolism of complex organisms by isolating the parts in question and growing them in artificial environments, that is to say, in "tissue cultures."

Why is the importance of this work not more generally recognized? Partly, as I have said, because it is so widely scattered. But perhaps also for another reason. If we review what I have said above one other fact is clear. Of all the "advances" which I have chosen to cite, not a single one was made with the 'classic' Carrel-flask technique. Seven were carried out with modifications of the hanging drop method, but only two of these, Harrison's and Carrel's (1907 and 1913) were made in the United States before 1940. All the rest are either recent—within the past decade—or made abroad, or made by methods other than the classic ones, or represent some combination of these. Only one—the demonstration of cellular immortality—came from Carrel's school of Tissue Culture in the grand manner which held the center of the American public stage for so many years. All the rest are from scattered independent laboratories, recent, and using less generally known methods. Perhaps they are not more widely recognized because they do not fall into an anticipated pattern, and because the public, even the scientific public, has not quite kept up with developments in the field. Newness, diffuseness and unexpected originality are not uncommon reasons for neglect. Hence the need for papers such as this.

In any case, Tissue Culture *is* an important tool, ready to the hand of anyone who can find a use for it. It is a versatile tool, capable of adaptation to a multitude of diverse tasks, as has been demonstrated over and over. Young biologists would do well to consider seriously the advantages of learning to use it effectively. It is *not* difficult to learn. Rigorous dis-

cipline and patience it does require, but so do most specialized techniques. And the rewards, to my mind at least, fully justify the effort.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

A METHOD FOR STUDYING SUCCESSIVE BATCHES OF EGGS LAID BY TREATED OR CONTROL DROSOPHILA*

Successive batches of eggs laid by female *Drosophila melanogaster* represent cells which on the average were at increasingly early meiotic and premeiotic stages at the time of treatment. The following technique[1] allows collection of almost all eggs laid during the lifetime of female flies. The effect of a given treatment upon fecundity and fertility can be studied. Furthermore, since the F_1 generation is obtained, by using appropriate genetic techniques studies can be made of hereditary changes (viable X-chromosome losses, chromosome rearrangements, recessive lethal mutations, mutations at specific loci, etc.) induced in the same sample of eggs in which hatchability has been determined. This advantage is of great importance in radiation genetics since one never obtains true estimates of the maximum mutation rates in females unless the rates for mature eggs are determined. The induced dominant lethal rate in these eggs is at least four times higher than that of oocytes in egg chamber located more distally in the ovarioles. Thus cells most sensitive to mutation give rise to a smaller proportion of the surviving offspring than do less sensitive cells. It is only by characterizing cells by their dominant lethal rates and then determining the hereditary changes in dominant lethal-free cells of the same sample that this error can be eliminated.

Containers are made by cementing netting on the top and bottom surfaces of plastic hoops. The hoops are sawed from rigid, plastic tubing of appropriate diameter. The netting, Dacron marquisette No. 1848 (Wellington Sears Co., N. Y. C., N. Y.), is cemented to the hoop with Weldwood contact cement (Weldwood Co., N. Y. C., N. Y.). Flies are dumped into the container through a hole drilled through the side of the hoop and stoppered by a small cork. The netting used has rectangular openings (0.55 × 0.75 mm) which are too small to allow the flies to escape, but which do allow eggs deposited by the females to pass through.

Boiling hot Drosophila food (per cent wet weight: propionic acid-0.7, agar-1.6, rolled oats-1.6, Anheuser Busch strain G primary, dried yeast-1.6, powdered charcoal-1.9, Karo white corn syrup-5.7, Grandma's molasses-5.8,

*Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

[1]This technique represents a modification of the one described by J. D. Telfer (Am. Nat. 88 ('54): 117–8).

yellow cornmeal-6.6, and water-74.5) is poured into Petri dishes, and these are refrigerated until they are to be used. By pressing a plastic lattice into the food a grid is stamped on its surface. This procedure divides the surface of the medium into squares (8 mm on a side) and greatly facilitates counting the eggs laid thereon. The circular, plastic, stamping lattice is generally dipped in 80 per cent ethyl alcohol, and the Petri plates containing black medium are stamped just before they are to be used. After stamping the plates are allowed to drain for a few minutes. The alcohol treatment of the medium stimulates egg production by female flies and inhibits yeast growth on the surface of the plate long enough for egg counts to be made.

If large numbers of eggs are to be collected daily, 50 pairs of flies are kept in large hoops (7.6 cm o.d., 2 cm h, 0.6 cm wall), whereas if the daily egg production of single females is to be determined, smaller hoops are used (3.8 cm o.d., 1.3 cm h, 0.2 cm wall). Large hoops are placed on medium sized Petri dishes (9.5 cm o.d., 2 cm h), while small Petri dishes (5.4 cm o.d., 1.5 cm h) are used with small hoops.

The following procedure is followed: The hoops containing males and females are transferred daily to fresh Petri plates. Each egg laying period of 19 hours (at 25 °C in the dark) is followed by a rest period of five hours (at 21 °C in the light). A hoop is placed upon food at 2:00 p.m. and removed at 9:00 a.m. the next day. During the egg laying period the Petri plates with hoops are stored in fly-proof plastic boxes (the kind used to store vegetables in home refrigerators). The eggs laid on the medium are counted at a magnification of 10X using a hand talley. The Petri dish is then covered with Nylon organdy (Macy's, N. Y. C., N. Y.) and incubated at 25 °C. The rectangular openings in this mesh (0.12 × 0.15 mm) are too small to allow first instar larvae to escape. The marquisette covering the hoop is brushed with a nylon scrubbing brush to destroy any eggs adhering to it. At 2:00 p.m. the hoop is placed on a fresh Petri plate and the procedure repeated. Parental flies can be maintained for as long as forty days in this fashion.

A second count is made at 1:00 p.m. on the following day of the unhatched eggs (now 27 to 46 hours old). The organdy is replaced, fastened firmly around the Petri dish with a rubber band, and the dish is covered with its lid. The resulting culture is kept at 21 °C until the F_1 appears.

Water vapor tends to condense on the under surface of the Petri dish cover. If the water forms a seal around the rim of the plate, the larvae within soon suffocate. To prevent the formation of a water seal the cover is kept from touching the rim of the plate by inserting a wedge between the rim of the dish and its cover. Tongue depressors or stick applicators cut to the appropriate size serve very well for this purpose.

R. C. KING

BIOLOGY DEPARTMENT
BROOKHAVEN NATIONAL LABORATORY
UPTON, N. Y.
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PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

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Andrewartha, H. G., and L. C. Birch, 1954. The distribution and abundance of animals. 782 p., ill. $15.00. University of Chicago Press, Chicago.

The basic problem of the relation between an organism and its environment is whether or not the organism is present. Its presence or absence, and abundance when present, depends on a variety of factors both historical and contemporary. Two Australian ecologists, Andrewartha and Birch, have presented a stimulating and very important discussion of some aspects of this problem. In considering ecological phenomena they have made a heroic attempt to separate fact from fancy and hypothesis from dogma. It is gratifying to see how well they have succeeded in their book, which may well be the "classic" work in ecology of our generation.

In their analysis the environment is divided into 4 components: weather, food, other animals, and a place in which to live. The manner in which these influence the distribution and abundance of animals is analyzed in critical detail and finally a general theory of the distribution and abundance of animals is presented. No attempt is made to cover the entire field of ecology. Community studies, for example, are thought to contribute little to the problem. Most of the data discussed are for arthropods though the criterion for selection seems to be appropriateness rather than systematic position.

The concept of competition is given severe scrutiny. The authors feel that in many instances competition is accepted as an article of faith when, in fact, no competition can be demonstrated. According to them, "Competition undoubtedly occurs, even though it may not be so widespread as sometimes imagined" (p. 23). Their views on density-dependent factors can be stated briefly, "We conclude that 'density-independent factors' do not exist; and there is no need to attach any special importance to 'density-dependent factors'—" (p. 19).

This work should be of great interest to ecologists, insect physiologists, and to all interested in evolution.

JOHN A. MOORE

Bacq, Z. M., and Peter Alexander, 1955. Fundamentals of Radiobiology. 389 p., ill. $6.50. Academic Press Inc., New York.

This book presents a balanced, coherent account of our present state of knowledge of radiobiology written by a physical chemist and a biologist in collaboration.

Bastin, Harold, 1955. Plants without flowers. 146 p. Colored frontispiece and black-and-white photographs; drawings by Jane Burton. $6.00. Philosophical Library, New York.

Brues, Charles T., A. L. Melander, and Frank M. Carpenter, 1954. Classification of insects. 917 p. 1219 text figures. $9.00. Bulletin 108, Museum of Comparative Zoology, Harvard College, Cambridge, Mass.

Since childhood Brues and Melander have been close personal friends and collaborators, first in boyhood experiments and mischief and subsequently in the field of entomology. In 1915 they privately published a "Key to the Families of North American Insects" in response to the need of students and investigators who were confronted with the task of identifying insects. With the rapid expansion of scientific investigations that has occurred during the 20th century and because of the unique position that insects occupy in the fauna of the world, entomology has become one of the major fields of interest for students of systematics. The result has been that more than three-quarters of a million of insect species have been described and the number of new species being described each year is still large and unquestionably will continue to be so since competent observers estimate that there remain more species to classify than have been catalogued to date. Obviously with such a mass of species to deal with, it will be totally impossible to present under the cover of one book a complete guide or key to the classification. At the familial level, however, it is still both possible and feasible, and this is what the authors have done. In 1932 they expanded their original private publication to include not only the insects of the world but also other terrestrial arthropods. Now they have revised the 1932 edition and, by adding the contribution of Dr. Frank Carpenter, have also included the fossil insects in their "Classification of Insects."

The book is divided into three parts, i.e., (1) the living insects, (2) the living terrestrial arthropods other than insects, and (3) the fossil insects. The section on living insects contains keys for the separation of the various classes of the phylum Arthropoda, and for the orders of insects as well as keys for the families of the various orders. The class Insecta is divided into two sub-classes and 27 orders, and thus is treated in a conservative manner when compared to some authors such as Omer-Cooper who organized the insects into four subclasses and 33 orders, and even in comparison to their own 1932 edition in which they had 34 orders. The treatment of each order consists first of a detailed definition of the order,

followed by a key to the families, often including keys to the sub-families of the major families, and finally a detailed listing of the taxonomic literature pertaining to the order. The literature listings for the larger orders are subdivided according to suborders or even families depending on the relative prominence of the various categories. For all the orders of "higher" insects which possess larvae and also for the three orders Odonata, Plectoptera and Plecoptera which have aquatic nymphs, separate keys are given for both the adults and the developmental forms. Synonyms are listed for both the orders and the families. Accompanying the major families are short lists of the most prominent genera as well as the range of distribution of the groups. The last are given in very abbreviated but effective form. The type of key format is the same as that previously employed in the earlier edition and places the contrasting statements in juxtaposition to each other on the same page. For ease of use this type of construction is certainly the best although, as the authors readily admit, it sometimes obscures natural relationship. Since keys almost invariably are artificial to some extent, the advantages of this type of key offset the disadvantages incurred. In addition to the three major contributors, C. L. Remington aided in the sections on the primitive orders Thysanura, Entrotrophi and prepared the larval key for the Lepidoptera, while Kenneth Christiansen revised the key to the Collembola.

The terrestrial arthropods other than insects are divided into eight classes and each of these is treated in a manner parallel to the treatment of the class Insecta.

Carpenter's section dealing with the extinct families of insects is a welcome and valuable contribution to the edition. It concerns itself with the extinct families of both extinct and living orders of insects. Forty-four orders of extinct insects have been erected by various authors and are listed in chronological order. Of these Carpenter recognizes only ten as valid. The keys themselves are not intended "to provide a key for identifying a fossil insect to its family" but rather to illustrate the more obvious taxonomic characters available in insect fossils and the present day pattern of classification. It will be quickly seen that the characters available from the fossils are much more limited than those employed for the classification of living insects. Much of the available fossil material is of a fragmentary nature, collected, of course, from relatively few localities, scattered over various parts of the world, and there obviously must exist much difference of opinion as to the placement of many of the specimens. Some are so fragmentary in nature that the only category in which they can be placed with any assurance is that of *Insectae incertae sedis*. Appended to the various categories listed in the keys and other lists are abbreviations showing the duration in time of the various insects according to the present available information.

This book will be a necessary tool for all students interested in arthropodan classification. The beginner or non-systematist, however,

should be warned that modern keys encompassing such a vast scope as these do must of necessity depend upon characters that can be studied and utilized only with the aid of modern optical equipment, which in this case automatically means use of a stereoscopic microscope, preferably one possessing various magnifications.

In a day when the energies of most biologists are turned to experimental studies, it is often forgotten that the correct identification of the material studied is still one of the prime essentials for sound investigations. Few, indeed, are the individuals who are competent and willing today to render such valuable service to the scientific community as the authors of this magnum opus have done.

HERMAN T. SPIETH

Bulletin signaletique d'entomologie medicale et veterinaire. No. 5 (4eme Trimestre 1954). 2 parts, 180 p. Office de la Recherche Scientifique & Technique Outre-Mer, Paris 7, France.

Butler, Elmer G. (Editor), 1955. Biological specificity and growth. 233 p., ill. $5.00. Princeton University Press, Princeton, N. J.

Eleven papers presented by biologists at the Twelfth Growth Symposium of the Society for the Study of Development and Growth:

Ontogeny of the blood and related antigens and their significance for the theory of differentiation; A. M. Schechtman; Immunobiological approach to some problems of induction and differentiation, M. W. Woerdeman; Immunogenetics, M. R. Irwin; Enzyme development as ontogeny of specific proteins, S. C. Shen; Cellular differentiation and the development of enzyme proteins in plants, R. Brown and E. Robinson; Some problems of specificity in the sexuality of plants, John R. Raper; General aspects of immunological reactions with bacteria and protozoa, James A. Harrison; Specificity in the relationship between host and animal parasites, W. E. Taliaferro; Compatibility and noncompatibility in tissue transplantation, Harry S. N. Greene; Specificity in growth control, Paul Weiss; Specificity of nuclear function in embryonic development, Robert Briggs and Thomas J. King.

Buxton, P. A., 1955. Animal life in deserts. 176 p., ill. $4.25. St. Martin's Press, Inc., New York.

Colbert, Edwin H., 1955. Evolution of the vertebrates. 479 p., ill. $8.95. John Wiley and Sons, Inc., New York.

A new, freshly-conceived and readable text book of vertebrate paleontology. Of the 122 illustrations nearly all are new, most of them by Lois Darling.

Cole, William H. (Editor), 1955. Some physiological aspects and consequences of parasitism. 90 p., ill. $2.00. Rutgers University Press, New Brunswick, N. J.

Papers presented at the Eleventh conference on protein metabolism as follows: Studies on the cultivation of malaria parasites, William Trager; The protein metabolism of intracellular parasites, James W. Moulder; Intestinal physiology and the host-parasite relationship, Clark P. Read; Studies of the glycolytic enzymes of Schistosoma mansoni, Ernest Bueding; Studies in antibody formation, William H. Taliaferro; Leishmaniasis in the hamster, Leslie A. Stauber.

Davis, H. S., 1953. Culture and diseases of game fishes. 332 p., 55 textfigures. $5.00. University of California Press, Berkeley, California.

Ford, E. B., 1955. Moths. 266 p., with 77 photographs in color, 71 in black and white by S. Beufoy, 19 maps and diagrams. $7.50. The Macmillan Company, New York.

The center of interest of this clearly written and beautifully illustrated book is in the elementary mechanisms of evolution as elucidated by studies of the population genetics and ecology of British moths. It contributes impressively to the aims of the New Naturalist Series of which it is a part: "to interest the general reader in the wild life of Britain by recapturing the inquiring spirit of the old naturalists." Its observations are drawn from moths in nature aided by the newer methods of experimental genetics and ecology, some of which, such as measures of population size by capture, marking, release and recapture, were introduced by the author and guided by concepts originated by him such as balanced polymorphism under the control of natural selection. It provides so excellent a focus for the field naturalist, that all interested in insects in nature will find in it new meanings for old observations, while laboratory scientists will gain more complete and satisfying views of their problems by seeing them followed into the natural habitats in which evolutionary changes can be observed. The splendid photographs, appendix on classification, glossary, index and bibliography increase the usefulness of an excellently produced book of high scientific importance.

L. C. DUNN

Gregg, John R., 1954. The language of taxonomy, an application of symbolic logic to the study of classificatory systems. 70 p. $2.50. Columbia University Press, New York.

The classifying taxonomist executes during his work numerous logical operations. It is the endeavor of this volume to analyze these procedures, particularly those involving establishment of the hierarchy of higher categories, and to express them in terms of the set theory. It does not appear to be the intent of the author to make a contribution to

the theory of taxonomy. Practicing taxonomists who handle concrete objects in their daily work and describe visual characteristics will presumably find the abstract symbols and rigid theorems of the set theory not very appealing. Logicians, however, should find this sound introduction into the grouping procedures of taxonomy very useful.

E. MAYR

Hagan, Harold R., 1955. Embryology of the viviparous insects. 472 p., ill. $6.00. The Ronald Press Company, New York.

Hall, Thomas S., and Florence Moog, 1955. Life science. A college textbook of general biology. 502 p., ill. $6.50. John Wiley & Sons, Inc., New York.

Hare, Ronald, 1955. Pomp and pestilence. Infectious disease its origins and conquest. 224 p., $5.75. Philosophical Library, Inc., New York.

Harris, J. R., 1954. An angler's entomology. 268 p. 103 color photographs, 24 black and white photographs, 27 maps and diagrams. $5.00. Frederick A. Praeger, New York.

Hausman, Leon Augustus, 1955. The bird book. 159 p. Illustrated in black-and-white and 16 Audubon color prints. $2.50. Arco Publishing Company, Inc., New York.

Helmcke, J.-G., and H. v. Lengerken (Editors), 1954. Handbuch der Zoologie. Vol. 7 (first half) Sauropsida: Allgemeines-Reptilia. Walter De Gruyter & Co., Berlin W 35, Germany.

Hill, Robert, and C. P. Whittingham, 1955. Photosynthesis. 165 p., ill. $2.00. A Methuen monograph on biochemical subjects. John Wiley & Sons, Inc., New York.

Howie, J. W., and A. J. O'Hea (Editors), 1955. Mechanisms of microbial pathogenicity. Fifth symposium of the society for general microbiology. 333 p. $5.00. Cambridge University Press, New York.

The infection of one organism by another may be followed by disturbances of function or metabolism, toxaemia, visible lesions, and death of the host organism. The object of this Symposium is to focus attention on the processes and mechanisms whereby infecting organisms produce such effects in their hosts. Contributions include authoritative articles from international authorities on the pathogenicity towards man, animals and plants, of bacteria, fungi and protozoa.

Hume-Rothery, William, 1955. Electrons, atoms, metals and alloys. 387 p., 171 photographs and diagrams. $10.00. Philosophical Library, New York.

Kirkaldy, J. F., 1955. General principles of geology. 327 p., ill. $6.00. Philosophical Library, New York.

Lewis, Harlan, and Margaret Ensign Lewis, 1955. The genus Clarkia. University of California publications in botany, Vol. 20, No. 4, pp. 241-392. 28 text figures. $2.00 (paper). University of California Press, Berkeley, California.

Marinelli, W., and A. Strenger, 1953 and 1954. Vergleichende Anatomie und Morphologie der Wirbeltiere. Probelieferung, 17 p., ill. 1. Lieferung, 80 p., 65 illustrations from original drawings by M. Wimmer. Verlag Franz Deuticke, Vienna, Austria.

Marshall, N. B., 1954. Aspects of deep sea biology. 380 p., 130 figs, 4 color plates. $10.00. Philosophical Library, New York.

Some early evolutionists hoped that little modified descendants of primordial life may yet be found in the depths of the ocean. A jelly-like material from the ocean bottom was hopefully named *Bathybius haeckeli*, only to be shown to represent an inorganic precipitate. Exploration of the deep sea life has, nevertheless, revealed a fascinating world of living creatures which are intensely interesting to evolutionists because of the remarkable and often bizarre adaptations of these creatures to their peculiar environments. A great deal of information on deep sea life is ably summarized in the present volume. Most of the illustrations are excellent.

TH. DOBZHANSKY

Miller, Gerrit S., Jr., and Remington Kellogg, 1955. List of North American recent mammals. 954 p., $3.50 (paper). U. S. Government Printing Office, Washington, D. C. United States National Museum Bulletin 205, Smithsonian Institution.

Nicol, Hugh, 1955. Microbes and us. 272 p. 65¢. Pelican books (A326). Penguin Books, Inc. Baltimore, Md.

Noble, G. Kingsley. 1954. The biology of the Amphibia. Unabridged republication of the first edition with a new biographical note on the author. 577 pp., ill., frontispiece photograph. $4.95. Dover Publications, Inc.

A reprinting of a long out-of-print but still useful classic work. First published in 1931, it is a pity that it has never been brought up-to-date in the light of two decades of new and important research, particularly in relation to evolution and classification.

Sevag, M. G., Roger D. Reid, and Orr E. Reynolds (Editors), 1955. Origins of resistance to toxic agents. 471 p., ill. $12.00. Academic Press, Inc., New York.

Sinnott, Edmund W., and Katherine S. Wilson, 1955 (fifth edition). Botany: Principles and problems. 528 p., ill. $6.75. McGraw-Hill Book Company, Inc., New York.

Smith, Mary, 1955. Baba of Karo: A woman of the Moslem Hausa. Edited and with introduction and notes by M. G. Smith; preface by C. Daryll Forde. 299 p. $7.50. Philosophical Library, New York.

This most interesting personal document was taken down by the wife of an anthropologist working at the time among the Moslem Hausa of northern Nigeria. Its real author is an elderly woman of the tribe who, with an utter lack of self-consciousness and a certain sturdy, peasant good sense, tells us the story of her life. Within this necessarily limited perspective, the specialist will find much essential information about Hausa kinship, marriage and childbirth, economics, religion, etc.

The introduction and notes (by the "author's" husband), while well and conscientiously done from a professional point of view, are of a nature to intimidate the lay reader. This is a pity, for there is much here for him also. Exoticism is the last and least important of the impressions which this book makes. In fact, the mutedly tragic story of how Baba was forced by intrafamilial pressures and machinations to break up her completely happy second marriage, might equally well (though at greater length) have been told by Austen, Balzac, or Tolstoi. Just as interesting, in the light of the current debates on colonialism, is the account of the effects of British rule in the Hausa area. According to Baba's evidence, it transformed a land of random and lawless violence to one of relative peace and security. One is tempted to wonder how much freedom and self-determination are worth at this price.

S. P. DUNN

Stiles, Karl A., 1955. Laboratory explorations in general zoology. 292 p. $3.75. The Macmillan Company, New York.

A laboratory manual for college freshmen.

Symposium on genetics of population structure. 141 p. $2.30. Libreria Internazionale Garzanti, Palazzo Universitario, Pavia, Italy.

Eleven papers on population genetics (Drosophila, mice, Potentilla and Achillea, Clarkia and others) presented at a symposium held at Pavia, August, 1953. All papers and discussions are in English.

Symposium "25 years of progress in mamalian genetics and cancer" held in celebration of the 25th anniversary of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine—June 27-30, 1954. Published by Journal of the National Cancer Institute, Vol. 15, No. 3, December 1954. pp. 551-853.

This is a collection of 21 papers with introductory material and discussion of each paper distributed as follows: genetics 10, cancer problems 7, behavior 3. The central paper both in location and subject matter since it connects and gives a new view of future connections between genetics and the study of cancer is by G. D. Snell on "The enhancing effect (or actively acquired tolerance) and the histocompatibility-2 locus in the mouse." This gives briefly and clearly the facts of acquired tolerance, that is, the change induced in an animal, previously resistant to the growth of an implanted tumor, by which the tumor is enabled to grow progressively. The hypothesis is presented that a specific enhancer is the product of a specific gene-locus (H-2), that when lyophylized tissue containing this product is injected into a resistant animal, the enhancer is incorporated in the host's cells and prepares them to support the growth of a tumor with the same specific substance. Other papers deal with genetics and disease, formal and physiological genetics of the mouse, origins of cancer in endocrine organs genetic control of behavior and genetic techniques in the study of cancer. A very suggestive concluding paper on the gene concept is contributed by Sewall Wright who also summarizes some of the other papers.

L. C. DUNN

Szafer, Władysław, 1954. Pliocene flora from the vicinity of Czorsztyn (West Carpathians) and its relationship to the Pleistocene. 238 p., 10 figures, 20 plates. zl. 48, 80. Text in Polish. Wydawnictwa Geologiczne, Warsaw.

The non-human primates and human evolution, 1955. Symposium papers arranged by James A. Gavan, in memory of Earnest Albert Hooton, 1887-1954. 134 p. $3.50. Wayne University Press, Detroit.

Contents: Dedication, James A. Gavan; The importance of primate studies in anthropology, Earnest Hooton; Fossil primates in the new world, G. L. Jepson; The geologic history of non-hominid primates in the old world, Bryan Patterson; Comparative anatomy of new world primates and its bearing on the phylogeny of anthropoid apes and men, G. E. Erikson; Primate evolution from the viewpoint of comparative anatomy, D. Dwight Davis; A comparative functional analysis of primate skulls by the split-line technique, N. C. Tappen; Metric and morphologic variations in the dentition of the Liberian chimpanzee; Comparisons with anthropoid and human dentitions, E. L. Schuman and C. L. Brace; Tentative generalizations on the grouping behavior of non-human primates, C. R. Carpenter; Problems of mental evolution in the primates, Henry W. Nissen; The cultural capacity of chimpanzee, Keith J. Hayes and Catherine Hayes; Closing remarks, William L. Straus, Jr.

Trevor, J. C., C. Radhakrishna Rao, and Ramkrishna Mukherjee, 1954. The ancient inhabitants of Jebel Moya. 123 p. $7.50. Cambridge University Press, New York.

This is an analysis of the results of an archeological expedition to the southern Sudan undertaken nearly forty years ago by the late Sir Henry Wellcome for combined scientific and philanthropic purposes. A large mass of skeletal and cultural remains was shipped back to England, and extensive anatomical data were also recorded on the spot. Unfortunately the former have suffered great damage during the intervening years of storage and shipment from place to place, and the latter have largely been discarded by the present authors as unreliable. It is indeed regrettable that the authors were called upon to follow such a cold trail, and some fuller explanation should have been offered of how this came about.

I am not in a position to evaluate the statistical work embodied here, the sheer bulk of which is impressive. The authors conclude that the Jebel Moya population was originally intrusive and perhaps predatory, and interbred with an autochthonous group to produce an extremely mixed physical type, with racial affinities to West Africa. Their culture appears similar to that of the present-day Nuba and Fung Tribes.

Finally, and ineffectually as always, I protest the inflated price of technical books. In this mechanical age there must be some way of cutting costs so as not to work hardship on the professional public, which the last I heard, was not beset by any embarrassment of riches.

S. P. DUNN

Waksman, Selman A. (Editor), 1955. Perspectives and horizons in microbiology. 220 p. $3.50. Rutgers University Press, New Brunswick, N. J.

Wardlaw, C. W., 1955. Embryogenesis in plants. 381 p., ill. $7.00. John Wiley & Sons, Inc., New York.

White, Philip R., 1955. The cultivation of animal and plant cells. 239 p., ill. $6.00. The Ronald Press Company, New York.

Worth, C. Brook, and Robert K. Enders, 1955. The nature of living things. 198 p., ill. 35¢. A Signet Key Book Ks326. New American Library, N. Y.

ERRATUM

In "A Mechanism of Gene Interaction," by Bernard S. Strauss, p. 150,

$$(1 + Ki/I) \cong 0$$

should read

$$\left(1 + \frac{Ki}{I}\right) \cong 1$$

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